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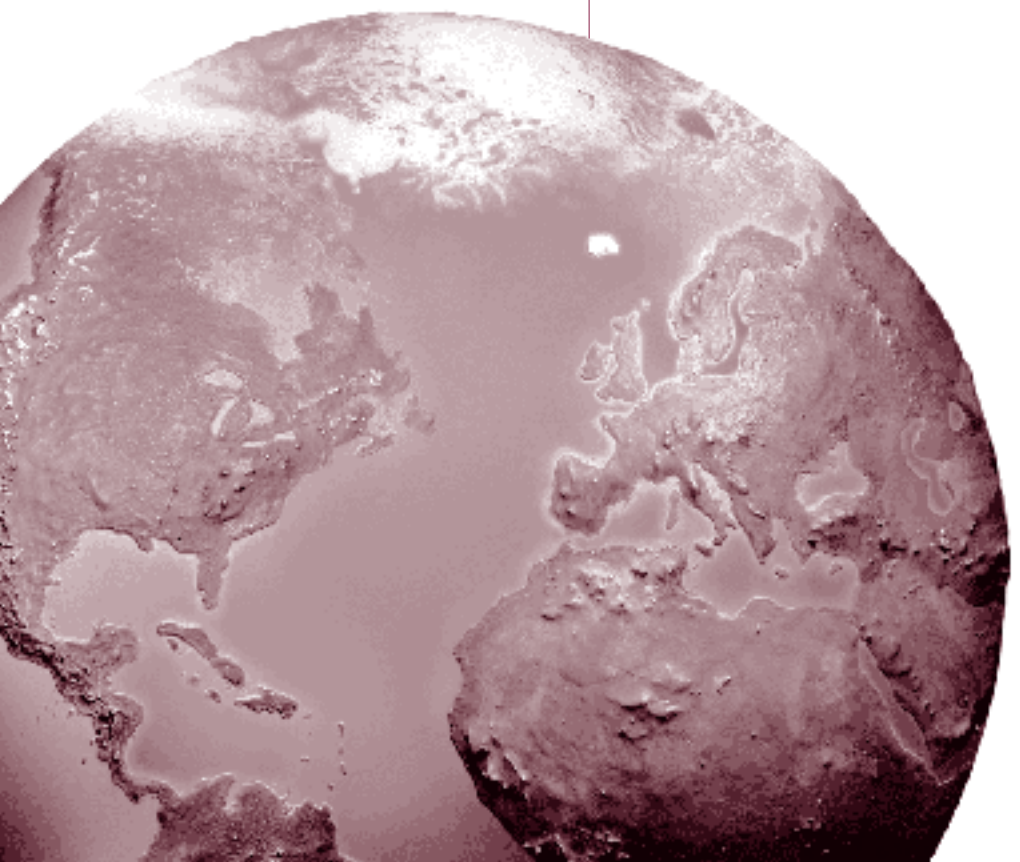
Uptake Distribution of Ozone in Human Lungs: Intersubject Variability in Physiologic Response

James S Ultman, Abdellaziz Ben-Jebria, and Steven F Arnold



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HEI STATEMENT

Synopsis of Research Report 125

Variability in Human Response to Ozone Uptake

Ozone is an irritant gas and a major component of smog. Some people exposed to ozone while undergoing moderate to strenuous physical activity experience reversible adverse responses in the lung (eg, irritation, inflammation, and decreased function). The degree of decreased lung function varies substantially among individuals exposed to the same level of ozone. Regulators need to know how the dose of ozone to the respiratory tract is related to the subsequent biological responses in order to estimate the health risks of ozone exposure.

Dr James Ultman and colleagues at Pennsylvania State University hypothesized that: (1) differences in ozone uptake in the lung are responsible for variation in decreased lung function and (2) variation in breathing patterns and lung anatomy among people is responsible for the different uptake. An instrument developed by these investigators in earlier HEI studies enabled quantitative determination of respiratory ozone uptake in exercising individuals.

APPROACH

Ultman and coworkers recruited 32 men and 28 women, all nonsmokers. The subjects first took a series of single breaths of air–ozone mixtures, which allowed the investigators to examine how ozone was distributed in the airways and where the major fraction of ozone was taken up. Two weeks later, the subjects pedaled a bicycle ergometer to produce conditions of moderate exercise for one hour while breathing clean air. Two weeks after that, they exercised under the same conditions while breathing ozone (0.25 parts per million). The investigators characterized each subject's lung anatomy before the 1-hour continuous air or ozone exposure. They computed the average tidal volume (the volume of air expired with each normal breath) and breathing frequency during each exposure. They also calculated the overall ozone uptake rate and the ozone uptake efficiency during exposure. Before each exposure and 10 and 70 minutes afterward, they measured standard parameters of lung function, including FEV_1 (forced expiratory volume in 1 second) and A_P, a novel measure of bronchial cross-sectional area available for gas diffusion that has been measured in few studies.

RESULTS AND INTERPRETATION

As expected, exposure to ozone caused a wide range of decreases in FEV_1 among the study subjects up to 70 minutes after exposure. These decreases did not correlate with ozone uptake rate, however, and thus did not validate the investigators' first hypothesis. In contrast, decreases in A_P after continuous ozone exposure correlated with ozone uptake rate, leading the authors to suggest that the hypothesis was validated. The use of A_P was a novel approach to evaluating changes in the transport properties of the lungs. However, because the physiologic significance of changes in A_P has not been validated, the strength of the investigators' conclusion is uncertain.

Ultman and colleagues found that the uptake efficiency of ozone was variable among the volunteers and lower in women (whose airways are generally smaller) than in men. This uptake efficiency was inversely correlated with breathing frequency and directly correlated with tidal volume in both sexes. Increased breathing frequency allows less time for ozone absorption during each breath; increased tidal volume drives ozone more deeply into the lung. These findings did validate Ultman's second hypothesis. They are consistent with the results of other investigators.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr James S Ultman at Pennsylvania State University, University Park PA. The following Research Report contains both the detailed Investigators' Report and a Critique on the study prepared by the Institute's Health Review Committee.

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CONTENTS

Research Report 125

Uptake Distribution of Ozone in Human Lungs: Intersubject Variability in Physiologic Response

James S Ultman, Abdellaziz Ben-Jebria, and Steven F Arnold

Departments of Chemical Engineering and Statistics, Pennsylvania State University, University Park, Pennsylvania

HEI STATEMENT

This Statement is a nontechnical summary of the Investigators' Report and the Health Review Committee's Critique.

INVESTIGATORS' REPORT

When an HEI-funded study is completed, the investigators submit a final report. The Investigators' Report is first examined by three ouside technical reviewers and a biostatistician. The report and the reviewers' comments are then evaluated by members of the HEI Health Review Committee, who had no role in selecting or managing the project. During the review process, the investigators have an opportunity to exchange comments with the Review Committee and, if necessary, revise the report.

CRITIQUE Health Review Committee

The Critique about the Investigators' Report is prepared by the HEI Health Review Committee and staff. Its purpose is to place the studies into a broader scientific context, to point out strengths and limitations, and to discuss remaining uncertainties and implications of the findings for public health.

Continued

RELATED HEI PUBLICATIONS

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INVESTIGATORS' REPORT

Uptake Distribution of Ozone in Human Lungs: Intersubject Variability in Physiologic Response

James S Ultman, Abdellaziz Ben-Jebria, and Steven F Arnold

ABSTRACT

The primary hypothesis of this study was that intersubject variation in uptake of inhaled ozone causes corresponding variation in the resulting physiologic response. The second hypothesis was that differences in breathing pattern and lung anatomy induce differences in ozone uptake.

Sixty healthy nonsmokers participated in three exposure protocols during which their minute ventilation was 30 L/min, corresponding to moderate exercise. For the intermittent bolus exposure to ozone (B_{O3}*), we measured the penetration volume at which 50% of the bolus was taken up ($VP_{50\%}$). Before and after continuous clean air exposure (C_a) and continuous ozone exposure (C_{O3}: 0.25 ppm ozone), we measured forced expiratory volume in 1 second (FEV_1), calculated as the percent change after exposure relative to start of exposure [$\%FEV_1$]). We also measured the cross-sectional area of the peripheral lung (A_P) for carbon dioxide (CO_2) diffusion, calculated as the percent change after exposure relative to start of exposure ($\%A_P$). After the C_{O3} session, we also measured ozone uptake (as ozone uptake rate) and fractional ozone uptake efficiency.

Uptake efficiency ranged from 0.70 to 0.98 among all subjects. It was inversely correlated with breathing frequency ($P = 0.000$) but was not correlated with conducting airways volume ($P = 0.333$). $VP_{50\%}$ ranged from 67 to 135 mL among all subjects and was directly correlated with conducting airways volume ($P = 0.000$). These results indicate that overall ozone uptake was related to breathing frequency but not to airway size, whereas internal distribution of ozone shifted distally as airway size increased.

Values of $\%FEV_1$ (mean ± SD: −13.71 ± 12.99) and $\%A_P$ (−7.80 ± 9.34) were both significantly more negative ($P = 0.000$) in the C_{O3} session than in the C_a (control) session (−0.055 ± 4.57 and 0.40 ± 11.03, respectively). Ozone uptake rate correlated with individual $\%A_P$ ($P = 0.008$) but not with individual $\%FEV_1$ ($P = 0.575$). Nor were individual $\%A_P$ or $\%FEV_1$ correlated with $VP_{50\%}$. Therefore, ozone uptake did not explain intersubject differences in forced expiratory responses in this study, but it did partially explain differences in the cross-sectional area available for gas diffusion in the peripheral lung.

INTRODUCTION

RATIONALE

The source of health effects from air pollutants is frequently described in terms of exposure response. In particular, when a potentially harmful gas is inhaled, it is distributed by flow and diffusion to tissues that undergo a local biological response in proportion to the dose received (US Environmental Protection Agency 1994). Ozone is a highly reactive, oxidant gas that is a common component of urban smog. As ozone is inhaled, it is distributed nonuniformly along the surface of the mucous blanket that lines the airway epithelium. Mucus contains antioxidants (such as uric acid, ascorbic acid, and glutathione) that can detoxify ozone (Cross et al 1994). Other substrates in mucus may react with ozone to form toxic products (eg, polyunsaturated fatty acids react with ozone to form aldehydes; Pryor et al 1996). The amount of either unreacted ozone or its toxic reaction products that reaches cells beneath the mucus surface is an important determinant of biological response. This response is further modulated by cellular processes (such as generation of inflammatory mediators or stimulation of irritant receptors) that may vary among cell types as well as among individuals.

Laboratory experiments have demonstrated marked differences in pulmonary response to acute ozone exposure

* A list of abbreviations and other terms appears at the end of the Investigators' Report.

This Investigators' Report is one part of Health Effects Institute Research Report 125, which also includes a Critique by the Health Review Committee and an HEI Statement about the research project. Correspondence concerning the Investigators' Report may be addressed to Dr James S Ultman, Department of Chemical Engineering, Pennsylvania State University, 106 Fenske Laboratory, University Park PA 16802-4400.

Although this document was produced with partial funding by the United States Environmental Protection Agency under Assistance Award R82811201 to the Health Effects Institute, it has not been subjected to the Agency's peer and administrative review and therefore may not necessarily reflect the views of the Agency, and no official endorsement by it should be inferred. The contents of this document also have not been reviewed by private party institutions, including those that support the Health Effects Institute; therefore, it may not reflect the views or policies of these parties, and no endorsement by them should be inferred.

among individuals (McDonnell 1996). These differences have important implications for risk assessment, which aims to protect susceptible human populations, but the reasons for intersubject variation in ozone exposure–response patterns are unclear. One problem in the interpretation of most clinical studies is that actual ozone uptake is not measured. Instead, a surrogate measure such as inhaled concentration or inhaled dose (ie, inhaled ozone concentration × ventilation rate × time) is often used as a predictor of the response. This practice leads to imprecise quantification of ozone uptake that can be misinterpreted as variation in response among individuals. The primary hypothesis of this study was that intersubject variation in pulmonary response is, in fact, related to individual differences in ozone uptake.

The reaction of ozone with antioxidants is so rapid (Kanofsky and Sima 1995) that there is little resistance to ozone uptake into the mucous layer. This fact implies that flow and diffusion of respired ozone is the limiting factor in its uptake. If that is the case, breathing pattern and airway anatomy should strongly influence ozone uptake and its longitudinal distribution along an airway. Thus, the second hypothesis of this study was that intersubject variation in breathing pattern and airway anatomy causes much of the intersubject variation in ozone uptake.

OZONE DOSIMETRY

Primarily because of a lack of suitable online gas analyzers, ozone uptake has rarely been measured. Wiester and colleagues (1996) measured overall ozone uptake into the lungs of 10 healthy men who breathed from a mask affixed to a large-diameter pipe through which ozonated air passed. Uptake was inferred from a decrease in ozone concentration along the pipe, and uptake efficiency was determined as the percentage of inhaled ozone taken up in the respiratory system. After measuring oral breathing at a ventilation rate of 10 L/min and an inhaled ozone concentration of 0.3 ppm, these investigators reported a mean uptake efficiency of 76.5% with great variation—51% to 92%—among subjects.

In a similar study of overall ozone uptake, 10 healthy nonsmokers inhaled 0.2 ppm or 0.4 ppm ozone through an oral mask while exercising continuously to elicit a minute ventilation of 20 L/min for 60 minutes or 40 L/min for 30 minutes (Rigas et al 2000). The researchers reported a mean overall uptake efficiency of 86% with a range of 80% to 91% among subjects. Thus, even under identical exposure conditions, individuals took up different amounts of ozone into their respiratory systems. This variation is a rationale for our first hypothesis: individuals experience differences in overall uptake that could account for corresponding intersubject variation in response.

To characterize the longitudinal distribution of ozone uptake into the intact human respiratory tract, we developed a method for introducing small ozone boluses into inhaled air at various times relative to the end of inspiration while respired flow and ozone concentration are continuously recorded at the lips (Hu et al 1992). Applying this method during oral breathing at a respired flow of 250 mL/sec (equivalent to a quiet ventilation rate of 7.5 L/min), we observed ozone uptake efficiencies of 60% in the upper airways and virtually 100% in the upper and lower conducting airways combined. At a respired flow of 1000 mL/sec (equivalent to an exercise ventilation rate of 30 L/min), uptake efficiencies dropped to about 10% in the upper airways and 90% in the conducting airways (Hu et al 1994). In addition to this distal migration of ozone caused by increasing ventilation, we found that individuals with larger volume of anatomic dead space take up ozone more distally than people with smaller dead space volume (Bush et al 1996). These observations are the source of our second hypothesis: differences in breathing pattern and airway anatomy induce differences in ozone uptake even among individuals who receive the same dose.

VARIABILITY IN PHYSIOLOGIC RESPONSE

Routinely gathering exposure–response data about a sufficiently large human population to characterize intersubject variation requires noninvasive measurements with high intrasubject reproducibility, such as FEV_1. In a landmark study of ozone exposure–response characteristics, McDonnell and coworkers (1983) performed forced spirometry during a 2.5-hour exposure of 135 healthy young men to ozone concentrations from 0.12 to 0.4 ppm while they exercised to elicit a ventilation rate of 65 L/min. For the subject population as a whole, decrements in FEV_1 were nonlinear functions of inhaled ozone concentration for exposures greater than 0.1 ppm. Equally important was the broad intersubject range in FEV_1 decrements that changed from −5% to +15% at 0.12 ppm ozone exposure to 0% to +45% at 0.40 ppm ozone.

Few studies have investigated age-related contributions to variation in ozone response. Drechsler-Parks and colleagues (1987) exposed healthy men and women to 0.45 ppm ozone for 2 hours during intermittent moderate exercise. The mean FEV_1 decrement was 5.8% for subjects aged 51 to 76 years compared with 17.8% for subjects aged 18 to 26 years. In a study based on data from healthy men aged 20 to 30 years, McDonnell and coworkers (1995) also demonstrated that the decrement in FEV_1 diminished with

age. Because functional residual capacity typically increases with age (Glindmeyer et al 1995), the attenuation of response in older individuals might be due in part to a reduction in their ozone uptake per unit area of lung surface.

Several investigations have focused on the possibility of sex-related contributions to variation in physiologic response. For example, Lauritzen and Adams (1985) exposed six women (aged 22 to 29 years) by mouth to ozone concentrations of 0.1 to 0.4 ppm during 1 hour of continuous exercise that elicited ventilation rates of 23 to 46 L/min. The observed decrements in FEV_1 were positively correlated with the inhaled dose, but the correlation was steeper than previously observed in men. The apparent sex difference was greatly attenuated when inhaled dose was normalized by maximum oxygen consumption. This result suggests that the different responses could have been due to a sex variation in ozone uptake that is inversely related to lung size. Adams and colleagues (1987) orally exposed 20 young men and 20 young women to 0.3 ppm ozone during 1 hour of continuous exercise that elicited a minute ventilation of 70 L/min in the men and 50 L/min in the women. The FEV_1 decrements of the men and women were about the same, possibly because the larger amount of ozone inhaled by the men was offset by a larger surface area over which the uptaken ozone was distributed.

A study comparing the physiologic response to ozone of four groups of young adults—white men, white women, black men, and black women—demonstrated a lack of sex and racial differences (Seal et al 1993). More than 90 subjects in each group were exposed to 0.12 to 0.4 ppm ozone for 2 hours while they intermittently exercised to elicit a minute ventilation of 25 L/min per square meter of body surface area. Within each group, decrements in FEV_1 were significantly correlated with ozone concentration, but interactions between the subject group and the FEV_1 decrements were not significant. Because lung size is roughly proportional to body surface area, the design of this study ensured that each subject received the same inhaled ozone dose per unit lung volume at each ozone concentration tested. The uniformity of response among the four groups further supports our contention that physiologic response is directly related to uptake, which is inversely related to lung size.

LOCALIZATION OF LUNG RESPONSE

Because ozone uptake is nonuniformly distributed between the airway opening and the distal lung, ozone uptake must be studied in the region of the respiratory system that mediates the response to the uptake. The identity of this target region may depend on the particular physiologic or biological endpoint that is used to define pulmonary response.

When characterized by lung mechanics, changes in pulmonary response typical of acute ozone exposure are parallel decreases in forced vital capacity (FVC) and total lung capacity with little change in residual volume, as well as an increase in airway resistance and a decrease in FEV_1 (McDonnell et al 1983). Inhalation of an anticholinergic aerosol (ie, atropine) by healthy young men before they were exposed to 0.4 ppm ozone prevented an increase in airway resistance but only partially blocked a decrease in FEV_1 (Beckett et al 1985). These observations suggest that mediation of mechanical responses by contraction of airway smooth muscle via cholinergic receptors greatly contributes to the ozone-induced increase in airway resistance but only partially explains the decrease in FEV_1. Atropine pretreatment did not prevent an ozone-induced decrement in FVC in that study, so another portion of the FEV_1 decrease may be due to inhibition of a full inspiratory effort.

Stimulation of neural C fibers has been suggested as another mechanism of mechanical response to acute ozone exposure. The increase in airway resistance was completely prevented by cooling the cervical vagal nerves of dogs exposed to ozone (Schelegle et al 1993). Direct measurement of vagal nerve impulses in dogs has further demonstrated that the C fibers are located in the lower conducting airways (Coleridge et al 1993). A reasonable assumption based on these studies is that decrements in forced spirometric parameters, whether originating from cholinergic receptors or C fibers, depend on ozone uptake in the lower conducting airways.

Because forced spirometry does not appear to elucidate the response of the peripheral portion of the lungs, other methods should be considered for this purpose. One logical candidate is the CO_2 expirogram. This noninvasive measurement contains a steep transition region (ie, phase II), from which conducting airways volume can be estimated, and a gently sloped plateau (ie, phase III) from which information about respiratory airspaces can be extracted (Figure 1). Following the work of Scherer and associates (1983), we assumed the source of the phase III slope to be an internal concentration stratification that facilitates CO_2 elimination by molecular diffusion. Because diffusion rate is proportional to the product of cross-sectional area and concentration stratification, A_P can be computed from the reciprocal of the phase III slope of the CO_2 expirogram (Appendix A).

SPECIFIC AIMS

The primary hypothesis of this study was that intersubject variation in the uptake of inhaled ozone causes

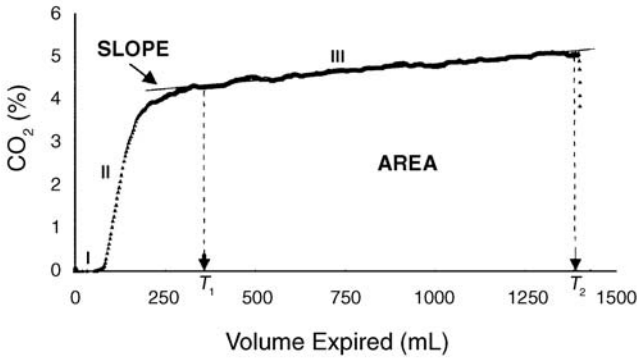

Figure 1. Sample CO_2 expirogram from one subject. The CO_2 expirogram consists of a pure dead space (phase I), a dead space transition region (phase II) and an alveolar plateau region (phase III). For test breaths, the SLOPE of phase III, the AREA under phase III, and the time difference (T_2-T_1) between the beginning and end of phase III were computed. Then the peripheral cross-sectional area, A_P, of the respiratory airspaces was computed as follows, using the diffusivity of CO_2 in air (D_{CO_2} = 0.16 cm^2/sec): $A_P = \{AREA/[D_{CO_2} \times SLOPE \times (T_2-T_1)]\}^{1/2}$.

corresponding variation in the resulting physiologic response. The second hypothesis was that differences in breathing pattern and lung anatomy induce differences in ozone uptake. The purpose of our study was to explore the influence of airway anatomy and breathing pattern on the relations among acute ozone exposure, overall ozone uptake, regional distribution of ozone uptake, and pulmonary response.

The specific aims of the study were to determine:

1. The relation of lung anatomy and breathing pattern to overall uptake of ozone.
2. The relation of lung anatomy to regional distribution of ozone.
3. The relation between overall ozone uptake and physiologic responses.
4. The relation between indicators of regional ozone uptake and response parameters.

METHODS

STUDY DESIGN

Experiments were carried out on 60 healthy nonsmokers, 32 men and 28 women. After a preliminary health screening, each subject participated in three types of exposure sessions—B_{O3}, C_a, C_{O3}—performed in that order (Table 1). The C_{O3} session for each subject was always conducted after the C_a session in order to maximize retention of subjects, especially those who were the most sensitive to ozone, and to minimize carryover of effects from ozone exposure. For the first 10 subjects, each type of session was duplicated in the following order: B_{O3}, B_{O3}; C_a, C_a; C_{O3}, C_{O3}. Thus, session-to-session differences within each subject could be compared to variation among subjects. For the remaining 50 subjects, the sessions were not duplicated.

Each session (whether a duplicate or not) was separated by two weeks or more for all subjects. All sessions were carried out at the Pennsylvania State University General Clinical Research Center under controlled temperature and humidity. All aspects of the study, including medical oversight, measurement procedures, and administration of informed consent, were approved by the Office of Research Protections of Pennsylvania State University.

During the first session (B_{O3}), distribution of ozone uptake was measured by the bolus inhalation method while subjects breathed at a respired flow of 1 L/sec. This flow corresponds to a minute ventilation of 30 L/min, typical of light to moderate exercise. During the second session (C_a), pulmonary responses were measured before and after subjects continuously breathed clean air and simultaneously pedaled a cycle ergometer for 1 hour at a workload that elicited a minute ventilation of 30 L/min. The third

Table 1. Summary of Experimental Protocol

Health screening session
Sign informed consent
Medical history → pregnancy test →medical examination
Spirometry → N_2 equilibration → exercise tolerance test
Bolus exposure (B_{O3})
Pregnancy test → vital signs → assessment questionnaire
Spirometry → acoustic reflection → CO_2 expirogram (preexposure)
50–100 breath bolus exposure→ vital signs (15-min intervals)
Continuous air exposure (C_a)
Pregnancy test →vital signs → assessment questionnaire
Spirometry → acoustic reflection → CO_2 expirogram (preexposure)
One-hour continuous air exposure → vital signs (15-min intervals)
Spirometry → CO_2 expirogram (10 min postexposure)
Spirometry → CO_2 expirogram (70 min postexposure)
Continuous ozone exposure (C_{O3})
Pregnancy test→ vital signs → assessment questionnaire
Spirometry → acoustic reflection → CO_2 expirogram (preexposure)
One-hour continuous ozone exposure → vital signs (15-min intervals)
Spirometry → CO_2 expirogram (10 min postexposure)
Spirometry → CO_2 expirogram (70 min postexposure)

session (C_{O3}) was conducted exactly as the second session except that the subjects breathed air containing 0.3 ppm ozone and overall ozone uptake was measured in addition to pulmonary response.

In each session, we characterized the subject's anatomy by using acoustic reflectometry to measure upper airways length and volume and by using CO_2 expirometry to measure conducting airways volume. During the C_a and C_{O3} sessions, pulmonary responses were quantified by changes in forced expiratory flow and volume (FEV_1, $FEV_{25\%–75\%}$, and FVC) obtained by spirometry as well as changes in A_P computed from CO_2 expirograms.

RECRUITMENT AND HEALTH SCREENING

The 60 young healthy nonsmokers (32 men and 28 women) were recruited without regard to race or ethnicity from the undergraduate students, graduate students, and staff of the University Park campus of Pennsylvania State University. We attempted to recruit equal numbers of men and women.

Initial screening was conducted by a combination of phone and office interviews. During these, each potential subject was given an explanation of the study, was required to sign an informed consent form, and was scheduled for a health screening at the General Clinical Research Center. During the health screening, the subject completed medical and smoking history forms. A physician then administered a medical examination, including height, weight, and blood pressure measurements and a resting electrocardiogram. Women were given a pregnancy test. A technician measured the subject's FEV_1, FVC, and forced expiratory flow between 25% and 75% of FVC ($FEF_{25\%–75\%}$) via a commercial spirometer that met American Thoracic Society criteria (110 Automated Spirometer, CDX Corp, Providence RI) and measured residual volume by nitrogen equilibration.

Each subject then completed an exercise tolerance test on a bicycle ergometer. During the test, a commercial apparatus (VMAX229 Legacy, SensorMedics, Yorba Linda CA) monitored the subject's electrocardiogram and recorded simultaneously oxygen consumption per unit time ($\dot{V}O_2$), minute ventilation, and pulse at graded exercise levels until maximum oxygen consumption per unit time ($\dot{V}O_2max$) was reached. A value of $\%\dot{V}O_2max@30$ was obtained from these data by expressing the $\dot{V}O_2$ measurement at 30 L/min as a percentage of $\dot{V}O_2max$.

A subject was only admitted into the study if he or she was not a smoker and did not have hay fever, asthma, chronic respiratory disease, or any other chronic disease. Also, the results of the spirometry administered during the screening had to be normal (FEV_1/FVC > 75% of the predicted Knudsen value [Knudsen et al 1976]). Subjects who regularly took medication of any kind except birth control pills or vitamin supplements were excluded from the study. (Of particular concern was regular use of antihistamines, decongestants, and antiinflammatory drugs, whether over the counter or prescription.) Women who were pregnant or trying to become pregnant were also excluded.

EXPOSURE SESSIONS

Medical Oversight

Immediately after arriving at the research center, each subject reported to the nursing staff to have a human chorionic gonadotropin (hCG) pregnancy test (women only), to have vital signs measured (ie, pulse rate, breathing rate, and blood pressure), and to complete a presession assessment questionnaire. The subject was not allowed to participate in the session if vital signs were abnormal or if the assessment revealed evidence of a respiratory illness within 24 hours or ingestion of antiinflammatory, decongestant, or antihistamine medications within 8 hours. These sessions were rescheduled.

After the subject was cleared by the medical staff to begin the session, preexposure values of dead space volume, A_P, FEV_1, FVC, $FEF_{25\%–75\%}$, and length and volume of the upper airways were measured. During each session, nursing staff recorded the subject's vital signs every 15 minutes. After each session, the subject remained in the research center until the nursing staff approved his or her discharge. Discharge was approved if the subject had no ozone-induced symptoms or if FEV_1 decrements had declined to minimal levels (ie, FEV_1 value close to its baseline).

B_{O3} Session

The purpose of the B_{O3} session was to determine the fractional uptake of ozone as a function of cumulative volume in the lungs by employing the bolus inhalation method (Hu et al 1992). At the start of each session, the subject sat at the bolus inhalation system and executed about three test breaths per minute. During each test breath, the subject began inhaling at functional residual capacity and then followed a triangular breathing pattern on the respired volume monitor. A breath was deemed acceptable if its mean inspiratory and expiratory flows were within 15% of the 1.0 L/sec target and if there was a minimal pause between inhalation and exhalation.

The timing of bolus injection was varied from breath to breath to cover uniformly bolus penetration volumes from 10 to 250 mL. To minimize systematic effects of time, this timing was varied in a random fashion. To ensure adequate resolution of the expired bolus concentration curves, a

peak inhaled bolus concentration of about 1 ppm was used. The B_{O3} session was terminated after 60 to 80 test breaths with acceptable breathing patterns were obtained.

C_a Session

The purpose of the C_a session was to determine the forced expiratory response due to exercise when the subject was breathing clean air. The procedure was identical to that followed in the C_{O3} session (described next) except that the UV lamp used to produce ozone in the inhaled air stream was turned off. The background concentration of ozone during the C_a sessions was less than 0.006 ppm, which is the resolution of the ozone analyzer (MacDougal et al 1998).

C_{O3} Session

The purpose of the C_{O3} session was to determine overall ozone uptake and the resulting physiologic response when exercising subjects were continuously exposed to ozone at a target concentration of 0.3 ppm (0.25 ppm actual) and a target minute ventilation of 30 L/min.

To begin each session, the subject sat on a cycle ergometer facing the continuous inhalation system, donned the breathing mask, and pedaled at a rate of 60 revolutions/min for 1 hour, following an audible cue from an electronic metronome. The ergometer workload was set at the value corresponding to $\%\dot{V}O_2max@30$ for that subject and was periodically adjusted throughout the session to maintain the target minute ventilation of 30 L/min as closely as possible.

Throughout the session, minute ventilation and inhaled ozone concentration were monitored by tracking the pneumotachometer and ozone analyzer outputs, respectively. Ten minutes after the session, dead space volume, A_P, FEV_1, FVC, and $FEF_{25\%-75\%}$ were measured again. These measurements were repeated a third time 70 minutes after the session.

MEASUREMENT METHODS

The instrumentation systems employed in this study were: (1) an automated spirometer used to measure FEV_1, FVC, and $FEF_{25\%-75\%}$; (2) a nitrogen equilibration system used to obtain residual volume; (3) a metabolic monitoring system used to obtain $\dot{V}O_2$, $\dot{V}O_2max$, and $\%\dot{V}O_2max@30$; (4) an acoustic reflectometer used to measure length and volume of the upper airways; (5) a CO_2 expirometer system used to determine dead space volume and A_P; (6) a bolus inhalation system used to measure the longitudinal distribution of ozone uptake; and (7) a continuous inhalation system used to measure the overall ozone uptake rate. Because they are not conventional instruments, the acoustic reflectometry, CO_2 expirometry, bolus inhalation, and continuous inhalation systems are discussed in more detail.

Acoustic Reflectometry

The geometry of the upper airways was characterized by using a commercial, acoustic reflectometer (ECCOVISION Pharyngometer, Hood Laboratories, Pembroke MA). This instrument measures airway cross-sectional area as a function of longitudinal distance by measuring the acoustic impedance of white noise generated near the lips. As previously described by Nodelman and Ultman (1999), the second relative minimum on a graph of the area–distance data was identified as the larynx, and the distance to this point was taken to be the length of the upper airways. The area–distance data were then integrated to determine the volume of the upper airways from the lips to the larynx. The nasal cavity was not included in these calculations because subjects breathed through mouthpieces during the B_{O3} session and through their mouths only in the C_a and C_{O3} sessions. Thus, the nose was never involved in respiration during the study.

CO_2 Expirometry

The CO_2 expirometer instrumentation system was developed specifically for this study to determine dead space volume (Fowler 1948) and A_P (Scherer et al 1983) from single-breath CO_2 expirograms obtained during quiet breathing. A particularly novel aspect of this project was use of this CO_2 expirometer to measure the change in A_P during exposure in order to quantify pulmonary responses in the respiratory airspaces.

When being tested by the CO_2 expirometer system, the subject sat in a chair and breathed through a mouthpiece assembly. CO_2 was monitored with an online capnometer (model 47210A, Hewlett-Packard, Palo Alto CA). Respiratory flow was simultaneously monitored with a pneumotachometer (Fleisch number 1). Subjects were first instructed to breathe normally and then were given audible cues from the experimenter. Subjects inhaled for three seconds and exhaled for three seconds for three sequential breathing cycles immediately followed by a fourth cycle during which they inhaled for three seconds and exhaled for five seconds. A data acquisition system (DAQ 801, Omega, Stamford CT) recorded the CO_2 and flow signals for these four breaths at a rate of 100 Hz.

In a computer program written to process these data, the first breath was disregarded and dead space volume was computed for the second and third exhalations by a method similar to that suggested by Heller and coworkers (1999). A_P was computed from the slope of and the area under a straight line fitted to the alveolar plateau of the fourth exhalation (Figure 1) according to equation A.2 (Appendix A).

The duplicate measurements of dead space volume and A_P for each of the first 10 subjects revealed that these parameters were statistically significantly affected by respired flow, which varied considerably for a given individual. Thus, a respired flow monitor was added to the apparatus and the remaining 50 subjects were trained to maintain a respired flow target of 250 mL/sec during both inhalation and exhalation.

Bolus Inhalation

The bolus inhalation system used during the B_{O3} sessions to determine the longitudinal distribution of ozone uptake is well described in previous publications (eg, Hu et al 1994). This apparatus originated from a breathing assembly that incorporated a pneumotachometer and a differential pressure transducer to monitor respired gas flow, an injection port to rapidly introduce a 20 mL ozone–air mixture (ie, a bolus), and a sampling port attached to a chemiluminescent ozone analyzer. This analyzer, developed with funding from HEI (Ultman et al 1997; MacDougal et al 1998), achieves the rapid response necessary for reliable ozone monitoring during rapid respiration conditions (such as those required for this study).

During each test breath in the B_{O3} sessions, a solenoid valve was triggered by a data acquisition system to automatically inject an ozone bolus into a subject's inhaled gas stream at a predetermined time relative to the end of inspiration. Ozone concentration and respiratory flow were continually recorded throughout the remainder of inspiration and the following expiration. In addition, the respired flow signal was continuously integrated to determine respired volume, which was displayed on a computer monitor in plain view of the subject. The subject was trained to match his or her breathing as closely as possible to the respired volume pattern that was predrawn on the monitor.

A complete experiment using this system required collection of a series of separate test breaths in which the bolus is injected earlier and earlier relative to the end of inhalation to measure ozone uptake deeper and deeper within the airways. After the experiment was complete, the data were analyzed by an automated computer program (designed in house) that cross-plotted the inhaled and exhaled ozone concentrations and respired volume data of each breath in order to determine the uptake fraction and penetration volume of an ozone bolus. The uptake fraction was computed as 1 minus the ratio of the integrals under the exhaled and inhaled concentration–volume curves; the penetration volume was computed as the centroid of the inhaled concentration–volume curve. After collecting and analyzing the entire set of 60 to 80 bolus test breaths per subject for which acceptable breathing patterns were obtained, the results were presented graphically as the uptake fraction versus penetration volume and characterized in terms of $V_{P_{50\%}}$ (Figure 2).

Continuous Inhalation

The breath-by-breath uptake of ozone during continuous exposure was measured by a minor modification of the continuous inhalation system we previously developed (Rigas et al 2000). This device originated as a two-way nonrebreathing valve with a common port, an inspiratory port, and an expiratory port. We connected the common port to a pneumotachometer fitted with an oral breathing mask and connected the inspiratory port to a continuous source of the desired ozone–air mixture. The expiratory port was exhausted into the room. The sampling line of our rapidly responding chemiluminescent ozone analyzer was connected between the breathing mask and the pneumotachometer.

The ozone–air exposure mixture was produced at 250 L/min by ultraviolet irradiation and input to one port of a piping tee. The second port of the tee served as an outlet and was connected to the inspiratory port of the two-way nonrebreathing valve. The third port of the tee served as a parallel outlet for ozonated air in excess of the subject's inspiratory demand. This excess air was vented to outside the system.

Ozone absorption by the continuous inhalation system itself was probably insignificant. All sections of the device were made of inert materials (such as stainless steel and

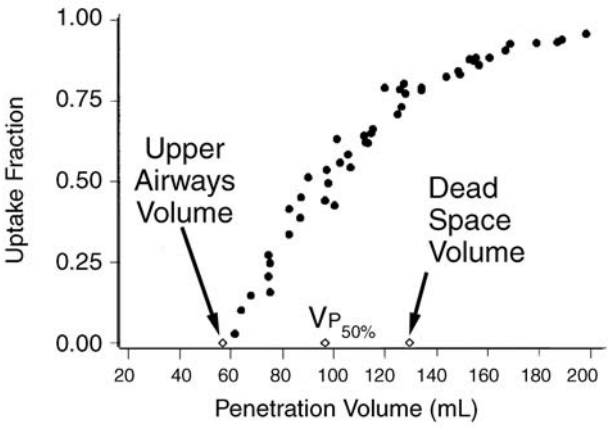

Figure 2. Sample ozone bolus distribution data from one subject. The penetration volume at which 50% of the inspired ozone becomes taken up, $V_{P_{50\%}}$, was computed by averaging the bolus penetration volumes of the 10 data points nearest (5 above and 5 below) an uptake fraction of 0.5. For this subject, no uptake occurred within the upper airways (where bolus penetration volume = upper airways volume) and the uptake fraction at the distal end of the conducting airways (where bolus penetration volume = dead space volume) was about 0.75.

Teflon) except the mask (silicone rubber) and some fittings (hard plastic). Although we did not test these materials in this study, we previously compared ozone absorption during oral breathing through a rubber mouthpiece, a hard plastic nasal cannula, and a bare stainless steel connection tube (Kabel et al 1994). Ozone absorption during breathing through these materials was negligible.

During the hour-long C_a and C_{O3} sessions, the voltage signals from both the chemiluminescent ozone analyzer and the pneumotachometer were recorded by a digital data acquisition system for the first 30 seconds of each minute. After each session, the flow and concentration data were numerically integrated to compute the time-averaged inhaled tidal volume, breathing frequency, minute ventilation, inhaled ozone concentration, and ozone uptake rate over each 30-second data acquisition period.

Instrument Calibration

All instruments were routinely calibrated at the beginning of each session. The acoustic reflectometer was calibrated by attaching a tube of known dimensions (supplied by the manufacturer) to the waveguide of the instrument. The online capnometer was calibrated by attaching a CO_2 standard cell (supplied by the manufacturer) to the optical sensor of the instrument. Pneumotachographs were calibrated by a known airflow that was simultaneously measured with a dry gas meter. The ozone analyzer was calibrated using a standard source approved by the US Environmental Protection Agency (49PS, Thermo Environmental Instruments, Franklin MA).

STATISTICAL METHODS AND DATA ANALYSIS

We were not always able to collect all data prescribed for exposure sessions for a particular subject. A session was considered complete when three conditions were met: (1) the subject finished the prescribed ozone exposure; (2) a complete set of continuous or bolus ozone uptake data were successfully recorded; and (3) FEV_1, FVC, and $FEF_{25\%–75\%}$ were measured at all predetermined time points.

The numbers of replicate measurements of dead space volume, A_P, FEV_1, FVC, $FEF_{25\%–75\%}$, and length and volume of the upper airways depended on the nature of the apparatus. For example, the commercial device used to measure FEV_1, FVC, and $FEF_{25\%–75\%}$ provided only one set of values at each time point, based on the subject's best effort. On the other hand, the acoustic reflectometer provided a set of four measurements of length and volume of the upper airways at each time point. When using the CO_2 expirometer, two sets of breathing maneuvers were conducted routinely so that four values of dead space volume and two values of A_P were measured at each time point. Unless stated otherwise, the replicate values of dead space volume, A_P, and length and volume of the upper airways were averaged at each time point to be comparable to the single values of FEV_1, $FEF_{25\%–75\%}$, and FVC. Moreover, the 30-second average measurements of the inhaled ozone concentration, ozone uptake rate, minute ventilation, tidal volume, and breathing frequency obtained in the continuous sessions were averaged over the 1-hour exposures in order to obtain a single set of values for each session.

Generally speaking, four types of statistical analyses were employed to analyze the anatomic, dosimetric, and physiologic response variables. Pearson product-moment correlations were employed (and Pearson product-moment correlation coefficient [Pearson r] values calculated) to determine the degree to which pairs of continuous variables were related, and best subsets regressions were used to simultaneously evaluate the influence of more than one continuous predictor variable. Analysis of variance (ANOVA) was applied to determine the significance of subject as random variable nested in sex, sex as a fixed variable, ozone or air exposure as a fixed variable, and session replicate as a fixed variable (Minitab software; Minitab, State College PA). Intersubject variation expressed as a percentage of total variation was calculated by employing a components-of-variance analysis as part of each ANOVA. To improve interpretability, a series of t tests was sometimes performed instead of a single ANOVA. In these analyses, equality of variance and equality of normality were confirmed with residual plots, and P values less than 0.05 were considered statistically significant.

RESULTS

FIRST 10 SUBJECTS

For the first 10 subjects who completed this study, the B_{O3}, C_a, and C_{O3} sessions were performed twice (in the sequence of B_{O3}, B_{O3}, C_a, C_a, C_{O3}, C_{O3}) to assess measurement repeatability. Comparison of these duplicated data (Figure 3) indicated that FEV_1 values from the C_{O3} session had excellent repeatability both before and after ozone exposure. Greater session-to-session variation in A_P values was due to subjects' lack of control of respiratory flow while recording the CO_2 expirograms. Respiratory flow changed as much as 250 mL/sec from measurement to measurement, causing variation in A_P of about 1000 cm^2. Most subjects were able to control their flow within 250 ± 30 mL/sec, however, causing variation in dead space volume and A_P on the order of only ± 3%. Variation in the

means of these measurements was even less. Because it was small relative to intersubject variation, the variation due to changes in flow was not included in calculations of dead space volume and A_P.

Figure 3 also indicates that $VP_{50\%}$ values from the B_{O3} sessions and fractional ozone uptake efficiency values from the C_{O3} sessions were consistently lower during the second session than during the first. We doubt that this result is due to a carryover of ozone exposure effects because the sessions were at least two weeks apart.

Quantitative comparison of the duplicate sessions was implemented by a series of ANOVAs (Table 2). Consistent with Figure 3, session-to-session differences in the response measurements $\%FEV_1$ and $\%A_P$ were not significant, whereas fractional ozone uptake efficiency values and $VP_{50\%}$ were significantly lower in session 2 than in session 1.

An important feature of this analysis was the use of intersubject variation expressed as a percentage of total variation to characterize the importance of between-subject variation compared with the sum of between-subject and within-subject variation. The purpose of our research, to determine the source of between-subject variation in ozone sensitivity, is best served when intersubject variation expressed as a percentage of total variation is as close

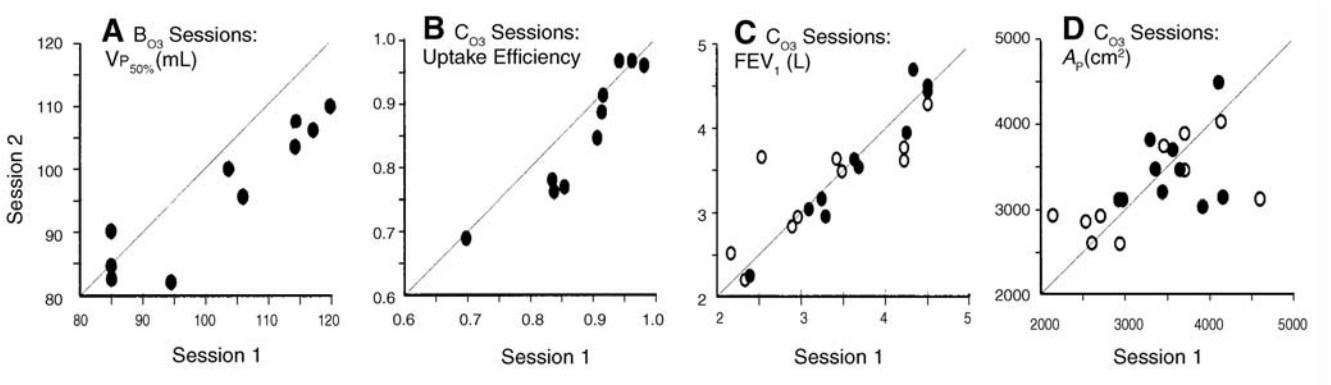

Figure 3. Day-by-day repeatability data for the first 10 subjects. Dosimetry parameters from the B_{O3} session (***A***) and the C_{O3} session (***B***). ***C*** and ***D***: response parameters from the C_{O3} session immediately before exposure (●) and 10 minutes after (○). The lines of identity correspond to perfect repeatability.

Table 2. Results of ANOVA from the First 10 Subjects[a] for B_{O3}, C_a, and C_{O3} Sessions

	$VP_{50\%}$ (B_{O3})	$\%FEV_1$[b] (C_a)	$\%FEV_1$[b] (C_{O3})	$\%A_P$[b] (C_a)	$\%A_P$[b] (C_{O3})	Uptake Efficiency (C_{O3})
Subject						
P	0.001	0.107	0.063	0.137	0.013	0.001
Intersubject variation (%)[c]	83.6	41.1	50.0	36.6	66.8	82.9
Session						
P	0.008	0.567	0.506	0.387	0.741	0.023
Mean[d]	102.4/96.1	0.231/−0.532	−11.00/−8.26	−1.02/−6.04	−8.29/−6.92	0.885/0.854
Sex						
P	0.044	0.382	0.839	0.701	0.801	0.007
Mean[e]	91.9/106.7	0.771/−1.072	−8.91/−10.34	−1.92/−5.15	−6.43/−8.78	0.803/0.936
N[f]	20	20	20	20	20	20

[a] Mean subject characteristics for 5 men and 5 women: 23 years, height 170 cm, weight 65.7 kg.

[b] Response computed as 100[(10 min postexposure)−(preexposure)]/(preexposure).

[c] Variance explained by subject (nested in sex) as a percent of total variance.

[d] Means for session 1/session 2.

[e] Means for women/men.

[f] Total observations for the 10 subjects.

Table 3. Mean Characteristics of the Study Subjects[a]

	Women	Men	All
60 Subjects Who Completed the Study			
N	28	32	60
Age (yr)	22.4 ± 0.9	22.9 ± 0.8	22.6 ± 0.6
Height (cm)	166 ± 1	178 ± 1	172 ± 1
Weight (kg)	62.1 ± 2.2	80.6 ± 2.5	72.0 ± 2.0
Residual volume (L)	1.11 ± 0.05	1.38 ± 0.06	1.26 ± 0.05
FEV_1/FVC (%)	84.1 ± 0.9	81.3 ± 0.9	82.6 ± 0.7
$\dot{V}O_2max$ (L/min)	2.09 ± 0.09	3.22 ± 0.10	2.69 ± 0.10
$\%\dot{V}O_2max@30$[b]	53.5 ± 1.5	39.4 ± 1.2	46.0 ± 1.3
47 Subjects Coached[c] on CO_2 Expirogram			
N	23	24	47
Age (yr)	22.30 ± 0.90	23.08 ± 0.99	22.70 ± 0.66
Height (cm)	166.49 ± 1.52	178.23 ± 1.53	172.49 ± 1.37
Weight (kg)	62.86 ± 2.52	83.00 ± 2.93	73.14 ± 2.43
Residual volume (L)	1.08 ± 0.05	1.33 ± 0.07	1.20 ± 0.05
FEV_1/FVC (%)	83.96 ± 1.06	81.21 ± 1.03	82.55 ± 0.76
$\dot{V}O_2max$ (L/min)	2.10 ± 0.11	3.36 ± 0.11	2.74 ± 0.12
$\%\dot{V}O_2max@30$[b]	53.10 ± 1.71	37.55 ± 1.24	45.16 ± 1.55

[a] Data are means ± SEs.

[b] $\dot{V}O_2$ at 30 L/min as a percentage of $\dot{V}O_2max$.

[c] Coached to breathe with a constant 250 mL/sec flow during the CO_2 expirogram maneuver.

Table 4. Results of ANOVA of Preexposure Data Combined for B_{O3}, C_a, and C_{O3} Sessions

	FEV_1[a] (L)	A_P[b] (cm^2)	Dead Space Volume[b] (mL)	Upper Airways Volume[a] (mL)
Subject				
P	0.000	0.000	0.000	0.000
Intersubject variation (%)[c]	89.5	40.8	67.5	73.9
Sex				
P	0.000	0.001	0.000	0.893
Mean[d]	3.22/4.38	3384/3786	132/160	51.2/51.6
N[e]	180	141	141	180

[a] FEV_1 and upper airways volume were analyzed for the 60 subjects who completed the study.

[b] A_P and dead space volume were analyzed for the 47 subjects coached to breathe with a constant 250 mL/sec flow during the CO_2 expirogram maneuver.

[c] Variance explained by subject (nested in gender) as a percentage of total variance.

[d] Means for women/men.

[e] Total observations: subjects × sessions.

to 100% as possible. In fact, the percent intersubject variation for $\%FEV_1$, for uptake efficiency, and for $V_{P_{50\%}}$ was 50% or more during ozone exposures, indicating that duplication of sessions was less important than maximizing the number of subjects who participated in the study. Percent intersubject variation was less than 50% for $\%A_P$, but this problem was overcome in the remaining 50 subjects by training them to maintain respiratory flow at 250 mL/sec during CO_2 expirogram maneuvers.

Thus, for each of the remaining 50 subjects, only one session of each type was conducted. In the final data analyses, the first set of sessions obtained from the first 10 subjects was combined with the single sessions for the additional 50 subjects.

FINAL DATA ANALYSIS

Preexposure Data

Of the 89 subjects scheduled for a health screening, 19 did not participate in any exposure sessions and 10 participated in one session before deciding not to participate in further sessions. The remaining 28 women and 32 men participated in all three exposure sessions and all dosimetry and forced expiratory data were recorded. We considered these 60 subjects to have completed the study. We did not notice any difference between these subjects and the 10 subjects who only appeared for a single exposure session.

Of the subjects who completed the study, 51 were self reported as white, 5 as Asian, 3 as Hispanic, and 1 as black. The overall characteristics of these subjects are shown in Table 3. Only 47 of the 60 subjects (23 women and 24 men) completed all the coached CO_2 expirogram maneuvers. Therefore, in all analyses involving dead space volume and A_P, only the data for these 47 subjects were used. Their characteristics are also summarized in Table 3.

Table 4 describes results of the ANOVA of intersubject differences in the preexposure physiologic variables FEV_1 and A_P and the anatomic variables dead space volume and upper airways volume. These data were pooled for the B_{O3}, C_a, and C_{O3} sessions for a total of 180 values of FEV_1 and upper airways volume (for the 60 subjects who finished the study) and 141 values of A_P and dead space volume (for the 47 subjects who were coached to control their respired flow during CO_2 expirometry). All of the response variables exhibited intersubject differences. The significance of these values is demonstrated by the low *P*

values and their importance is shown by the relatively large values of intersubject variation expressed as a percentage of total variation. All predictor variables were significantly different by sex except volume of upper airways. This result is not surprising because FEV_1, A_P, and dead space volume reflect lung size but upper airways volume probably does not, and the men in this study had on average larger lungs than the women. For example, in this study the mean preexposure FVC for men (5.46 L) was significantly greater than that for women (3.82 L) ($P = 0.000$; data not shown).

Although not included in Table 4, analyses of the preexposure values of the remaining physiologic variables, FVC and $FEF_{25\%–75\%}$, yielded results that were similar to those for FEV_1. In fact, FVC and FEV_1 were strongly correlated (Pearson $r = 0.946$, $P = 0.000$) (Figure 4), as were $FEF_{25\%–75\%}$ and FEV_1 (Pearson $r = 0.714$, $P = 0.000$).

Using an apparatus and data analysis program of our design, dead space volume and A_P were determined using measurements from single-breath CO_2 expirograms. To assess the reliability of the dead space volume measurements, we compared our data to a relation of dead space volume with height previously reported by Hart and associates (1963). The curve produced by the Hart relation passes through the center of our data (Figure 5). The scatter of our data around the curve is roughly equivalent to that of Hart's own data (not shown). The correlations of dead space volume with height (Pearson $r = 0.568$, $P = 0.000$) and with respiratory system volume as characterized by FVC (Pearson $r = 0.585$, $P = 0.000$; data not shown) are similar, further supporting the plausibility of the dead space data.

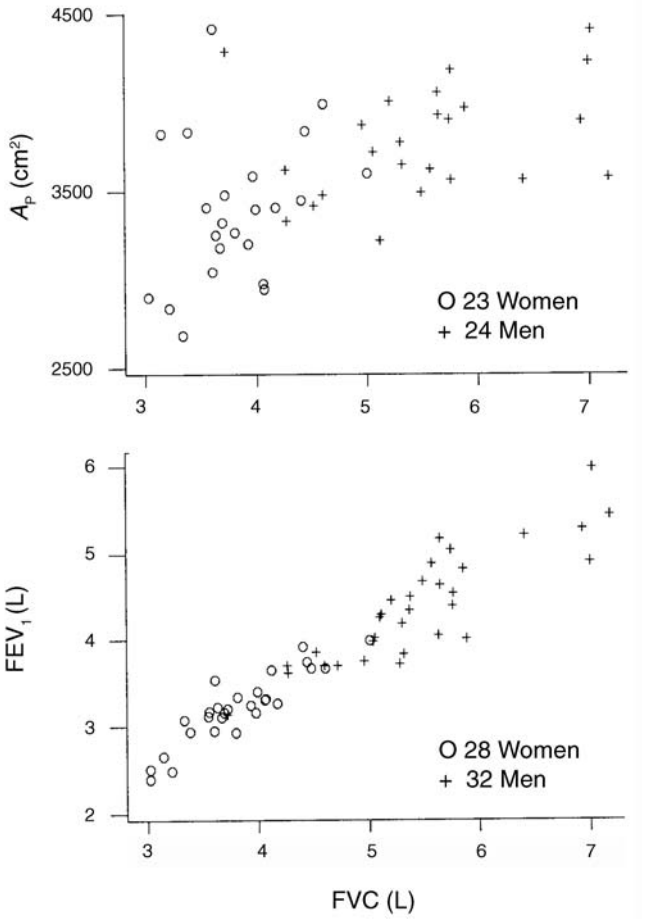

Figure 4. Relation of lung size (by FVC) on preexposure values of A_P and FEV_1. Both A_P (Pearson $r = 0.531$, $P = 0.000$) and FEV_1 (Pearson $r = 0.946$, $P = 0.000$) were correlated with FVC.

Dosimetry Variables

In both the C_{O3} and C_a sessions, the experimenter targeted a minute ventilation of 30 L/min by periodically adjusting ergometer workload. In fact, the mean ± SD of minute ventilation pooled for all breaths of all subjects in both the C_a and C_{O3} sessions was 29.9 ± 3.7 L/min. An ANOVA of the minute ventilation averaged over all breaths of each subject in each session indicated significant differences among subjects. However, neither minute ventilation between women and men nor minute ventilation between the C_a and C_{O3} sessions differed significantly (Table 5).

Whereas minute ventilation measurements did not differ systematically either between or within the C_a and C_{O3} sessions, a small but significant increase in breathing frequency and decrease in tidal volume was associated with the addition of ozone to inhaled air (Table 5). This ozone-induced change in breathing pattern became evident during the last half of the sessions (Figure 6). This finding supports the concept that people reduce their tidal

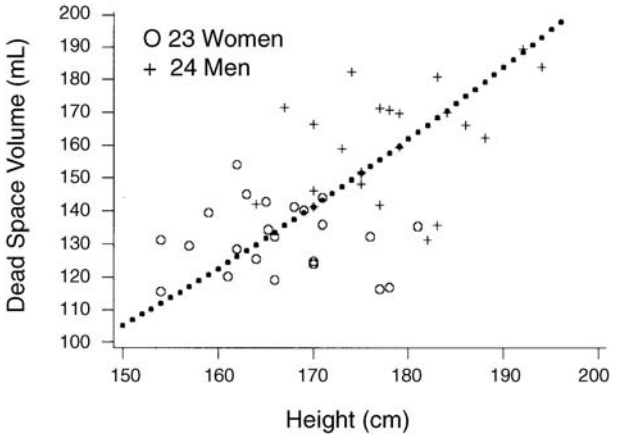

Figure 5. Anatomic dead space volume obtained from the CO_2 expirogram. Relation reported by Hart and associates (1963) is shown by the dotted curve. Dead space volume was correlated with height (Pearson $r = 0.568$, $P = 0.000$).

Table 5. Results of Analysis of Dosimetry Variables in C_a and C_{O3} Sessions

	Breathing Frequency[a] (bpm)	Tidal Volume[a] (L)	Minute Ventilation[a] (L/min)	Inhaled Ozone Concentration[b] (ppm)	Uptake Efficiency[b]
Subject					
P	0.000	0.000	0.000	—[c]	—
Intersubject variation (%)[d]	84.7	75.5	52.1	—	—
Session					
P	0.010	0.006	0.109	—	—
Mean[e]	29.3/30.4	1.10/1.04	30.6/29.8	—	—
Sex					
P	0.000	0.000	0.066	0.012	0.008
Mean[f]	32.7/27.0	0.93/1.21	29.4/31.0	0.242/0.261	0.871/0.914
N[g]	120	120	120	60	60

[a] Results of an ANOVA for the 60 subjects who completed the study.

[b] Results of an unpaired *t* test comparing the 28 women and 32 men who completed the study.

[c] — = not applicable.

[d] Variance explained by subject (nested in sex) as a percentage of total variance.

[e] Means for C_a session/C_{O3} session.

[f] Means for women/men.

[g] Total observations: subjects × sessions.

volume to avoid the discomfort associated with progressive exposure to ozone (McDonnell et al 1983). There was also a sex-related difference in breathing pattern: the women had a significantly higher breathing frequency and a significantly lower tidal volume than the men on average, probably because of the smaller lung volume of the women (Table 5).

In the C_{O3} session, the experimenter targeted an inhaled ozone concentration of 0.3 ppm by periodically adjusting ozone generation. Although inhaled ozone concentration varied minimally minute by minute within any 1-hour continuous exposure (data not shown), inhaled ozone concentration was consistently lower than its target (mean ± SD: 0.252 ± 0.029 ppm) and was significantly lower for the women than for the men (Table 5). These deviations from the target inhaled ozone concentration were due to the following time course: because of residual exhaled air in the breathing assembly and at the proximal end of respiratory tubing, ozone concentration rapidly rose at the beginning of inhalation, reaching a plateau that persisted until the end of inhalation. For practical reasons, the experimenter adjusted the inhaled ozone concentration by observing the plateau level, which was always higher than the flow-weighted time-averaged inhaled ozone concentration reported in Table 5. The differences in inhaled ozone concentration between subjects was a direct result of individual differences in tidal volume; the larger the volume, the longer the plateau in inhaled ozone concentration and the more accurate (ie, larger) the experimenter's estimate of inhaled ozone concentration.

The mean (± SD) fractional ozone uptake efficiency for the C_a and C_{O3} sessions combined for all subjects was 0.89 ± 0.06. For individual subjects, session-averaged uptake efficiencies were 0.70 to 0.98, with significant differences between men and women (Table 5). These intersubject differences were inversely correlated with breathing frequency and directly correlated with tidal volume (Figure 7). Uptake efficiency was also affected by exposure time. The mean (± SD) uptake efficiency among all subjects in four sequential 15-minute intervals during the 1-hour C_{O3} sessions were 0.906 ± 0.058, 0.903 ± 0.060, 0.895 ± 0.064, and 0.873 ± 0.088. An ANOVA of these data indicated that uptake efficiency decreased slightly but significantly ($P = 0.000$) as exposure time increased (ie, a mean 0.011 decrease per 15-minute interval). This result is consistent with our finding that the increasing breathing frequency and decreasing tidal volume that occurred during the C_{O3} session (Figure 6) led to a decrease in uptake efficiency (Figure 7).

Values of mean ozone uptake rate were equivalent to the product of minute ventilation, inhaled ozone concentration, and fractional ozone uptake efficiency; thus, intersubject variation in ozone uptake rate was driven by variation in

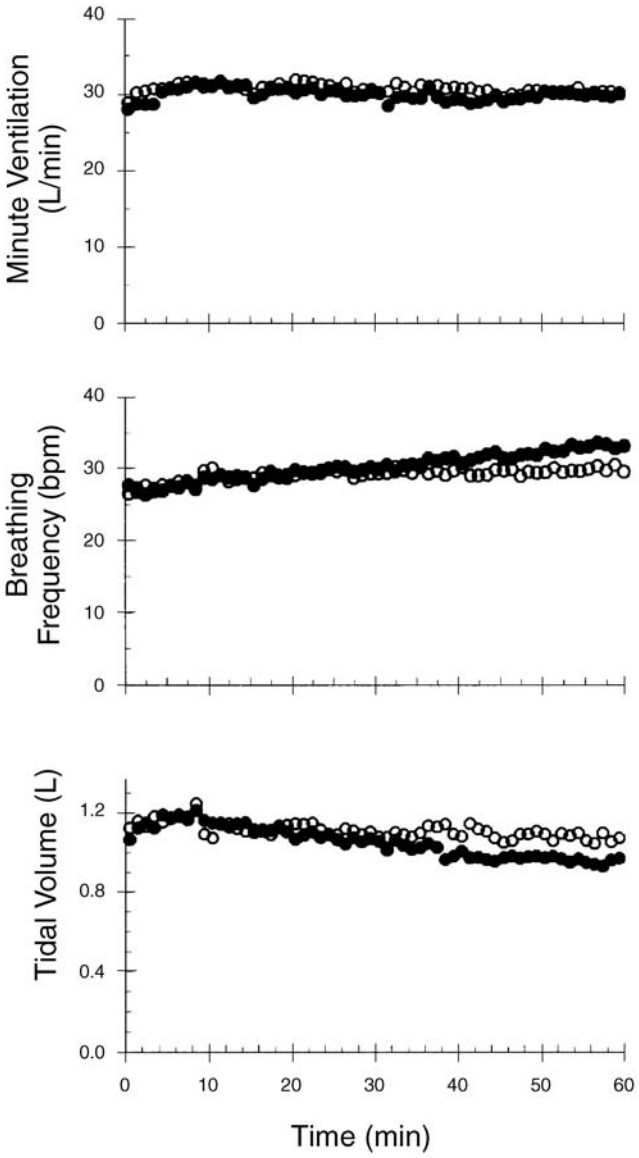

Figure 6. Minute-to-minute changes in breathing parameters during the hour-long C_a (○) and C_{O3} (●) sessions. Each data point represents the pooled values of all subjects' breath-by-breath data averaged over the 30-second data monitoring interval.

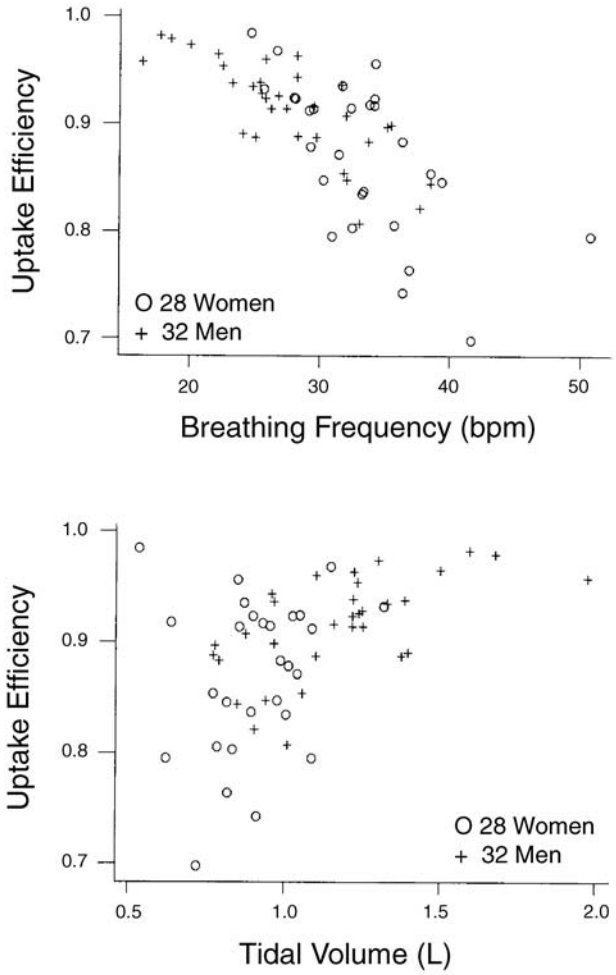

Figure 7. Ozone uptake in C_{O3} sessions. Uptake efficiency strongly depended on breath frequency (Pearson $r = -0.723$, $P = 0.000$) and on tidal volume (Pearson $r = 0.490$, $P = 0.000$).

three different variables. The minute ventilation, inhaled ozone concentration, and uptake efficiency values for the subject with the lowest ozone uptake rate (3.2 ppm-L/min) were 14 L/min, 0.23 ppm, and 0.98. For the subject with the highest ozone uptake rate (9.1 ppm-L/min), they were 31 L/min, 0.30 ppm, and 0.98. Thus, although we controlled minute ventilation and inhaled ozone concentration to the best of our ability, some subjects exhibited large variation in these variables, resulting in a wide range of ozone uptake rates (Figure 11).

During the B_{O3} session, the longitudinal distribution of ozone bolus uptake was determined while each subject controlled his or her breathing to follow a predrawn pattern on a computer monitor, which corresponded to a target respired flow of 1000 mL/sec. The actual respired flow for the 60 subjects was close to this value (mean ± SE was 952 ± 6 mL/sec during inhalation and 991 ± 6 mL/sec during exhalation). The distribution of ozone bolus uptake of a typical subject (Figure 2) indicates that, at the relatively high minute ventilation in this study, ozone boluses swept through the upper airways without being taken up (penetration volume < volume of upper airways) whereas a considerable amount of ozone reached the airspaces where respiratory gas exchange occurs (penetration volume > volume of anatomic dead space). In fact, for 56 of the 60 subjects, the volume of upper airways was less than the penetration volume at which measurable ozone occurred and, in all subjects, about

25% of the inhaled ozone penetrated beyond volume of anatomic dead space into the respiratory airspaces.

As a means of quantifying intersubject differences in ozone bolus uptake, $VP_{50\%}$ was computed for each uptake distribution. Individual values were 69 to 134 mL and were directly correlated with individual values of dead space volume (Pearson $r = 0.570$, $P = 0.000$). A somewhat improved relation was obtained by correcting the correlation for the fact that uptake did not occur within the upper airways volume by subtracting this volume (Figure 8). A two-sample t test indicated that the mean penetration volume at which 50% of the bolus was taken up for the women (90.4 mL) was significantly smaller ($P = 0.000$) than that for the men (107 mL). In addition, $VP_{50\%}$ minus upper airways volume was directly related to dead space volume minus upper airways volume (Figure 8). When coupled, these results suggest that the larger surface-to-volume ratio associated with the smaller airways in women enhances local ozone uptake, thereby reducing the distal penetration volume of O_3 into the female respiratory system.

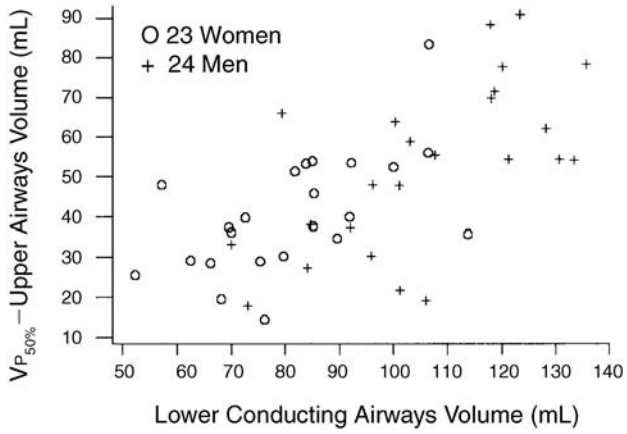

Figure 8. Ozone distribution during B_{O3} sessions. Penetration volume distal to the upper airways at which 50% of an ozone bolus was taken up ($VP_{50\%}$ − upper airways volume) was correlated with the volume of the lower conducting airways (dead space volume − upper airways volume) (Pearson $r = 0.650$, $P = 0.000$).

Physiologic Responses

Physiologic responses were quantified by percent changes after exposure relative to before exposure for the spirometric parameters ($\%FEV_1$, %FVC, and $\%FEF_{25\%-75\%}$) and the CO_2 expirometry parameter ($\%A_P$). Responses were measured 10 minutes and 70 minutes after the C_a and C_{O3} sessions. Because $\%FEV_1$ correlated highly with corresponding values of %FVC (at 10 minutes, Pearson $r = 0.945$, $P = 0.000$; at 70 minutes, Pearson $r = 0.903$, $P = 0.000$) and $\%FEF_{25\%-75\%}$ (at 10 minutes, Pearson $r = 0.937$, $P = 0.000$; at 70 minutes, Pearson $r = 0.903$, $P = 0.000$), the three spirometric variables were not independent indicators of physiologic response. Moreover, %FVC measured after the C_{O3} session did not correlate with mean ozone uptake rate. Therefore, $\%FEV_1$ is the only spirometric variable discussed further.

The physiologic responses to the C_a and C_{O3} sessions are shown in Table 6. Compared with inhalation of air (C_a), inhalation of ozone (C_{O3}) caused near-significant negativity of the mean $\%FEV_1$ and $\%A_P$ for women and men at 10 minutes and at 70 minutes after. The negativity of $\%FEV_1$ and in $\%A_P$ measured during the C_{O3} session were

Table 6. Results of Paired t Tests Comparing Physiologic Responses in C_a and C_{O3} Sessions[a]

	$\%FEV_1$[b]				$\%A_P$[c]			
	Men		Women		Men		Women	
Minutes postexposure	10	70	10	70	10	70	10	70
C_a								
Mean	−0.48	3.30	0.43	0.60	−2.10	−2.40	3.00	2.90
SE	0.96	1.80	0.65	0.62	2.30	2.10	2.30	3.00
C_{O3}								
Mean	−15.90	−12.30	−11.20	−9.35	−8.83	−7.90	−6.73	−4.05
SE	2.70	1.80	1.90	1.40	1.90	1.80	2.00	1.80
P	0.000	0.000	0.000	0.000	0.026	0.051	0.003	0.054
N[d]	64	64	56	56	48	48	46	46

[a] Responses computed as 100[(minutes postexposure − preexposure)/preexposure].

[b] For the 32 men and 28 women who completed the study.

[c] For the 24 men and 23 women who were coached to breathe with a constant 250 mL/sec flow during the CO_2 expirogram maneuver.

[d] Total observations: subjects × sessions.

greater in men than in women, corresponding to the higher ozone uptake (ie, the product of uptake efficiency and inhaled ozone concentration) for men compared with women (Table 5). However, results of a two-variable *t* test showed that the influence of sex on $\%FEV_1$ and $\%A_P$ was not statistically significant ($P > 0.16$). The $\%FEV_1$ and $\%A_P$ values after the C_a and C_{O3} sessions were not correlated (Figure 9).

A_P and FEV_1 values measured before exposure were strongly correlated (Figure 10, top panel) whereas $\%FEV_1$ and $\%A_P$ after the C_a and C_{O3} sessions were not (Figure 10, bottom panel). The relation between the preexposure values is logical because both A_P and FEV_1 depend on lung size (as characterized by preexposure values of FVC; Figure 4). The fact that the responses of these parameters to ozone exposure are unrelated suggests that $\%FEV_1$ and $\%A_P$ measure independent changes in lung function. This suggestion is further supported by the observation that the standard errors of the $\%FEV_1$ distribution among subjects were lower for the C_a session than for the C_{O3} session, whereas the opposite was true for $\%A_P$ (Table 6).

Uptake–Response Relations

In the first stage of analysis, we considered the effect of overall ozone uptake on $\%FEV_1$ and $\%A_P$. At 10 minutes after exposure, $\%A_P$ and ozone uptake rate were significantly correlated whereas $\%FEV_1$ and ozone uptake rate were not (Figure 11). This result supports the conclusion that $\%A_P$ and $\%FEV_1$ reflect ozone-induced changes in different aspects of pulmonary function. $\%A_P$ at 10 minutes after exposure was not significantly correlated with ozone inhalation rate (Pearson $r = -0.263$, $P = 0.074$), however,

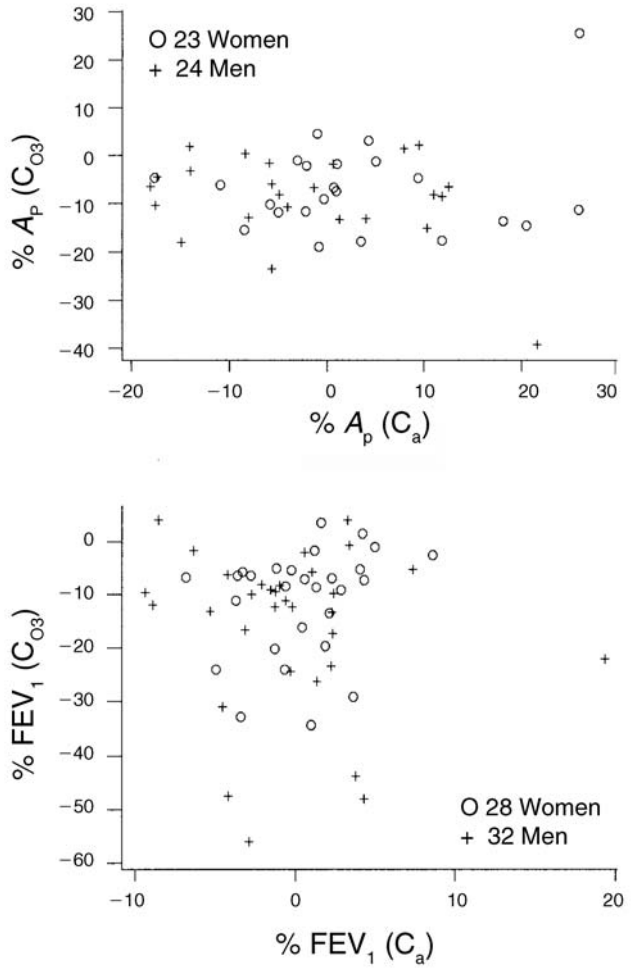

Figure 9. Physiologic responses to the C_a and C_{O3} sessions. Responses were computed as 100[(10 minutes postexposure − preexposure)/preexposure]. For $\%A_P$, Pearson $r = -0.034$ ($P = 0.821$); for $\%FEV_1$, Pearson $r = -0.006$ ($P = 0.965$).

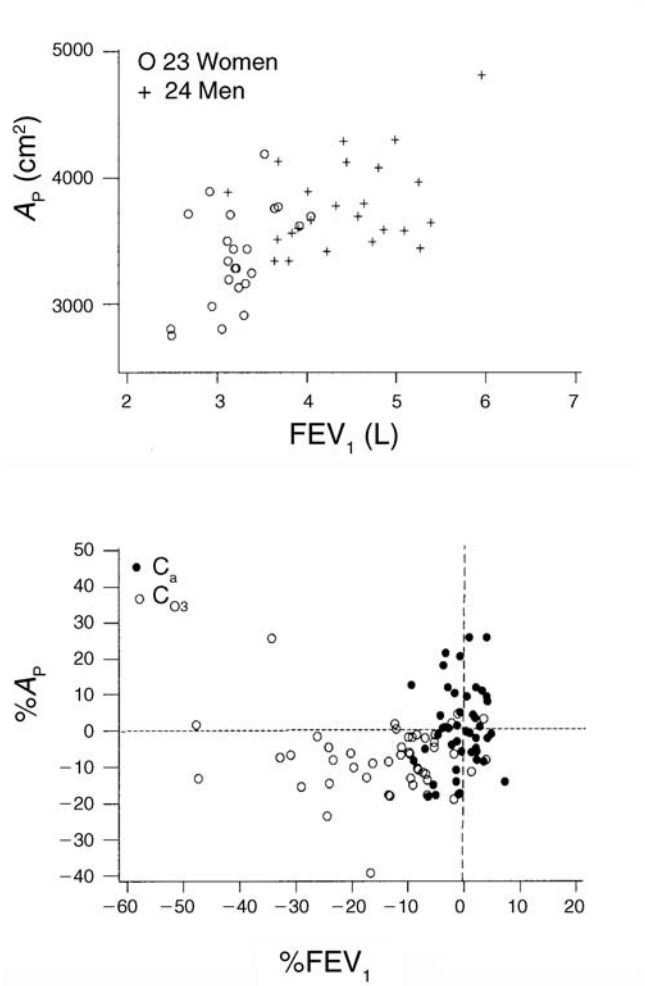

Figure 10. Responses of the 47 subjects coached on the CO_2 expirogram. Top panel: preexposure values of A_P and FEV_1 were correlated (Pearson $r = 0.606$, $P = 0.000$). Bottom panel: $\%A_P$ and $\%FEV_1$ were not correlated for C_a (Pearson $r = 0.109$, $P = 0.465$) or for C_{O3} sessions (Pearson $r = 0.000$, $P = 0.924$). All values were computed as 100[(10 minutes postexposure − preexposure)/preexposure].

indicating that inhaled ozone dose is a poorer predictor of the A_P response than is ozone uptake. At 70 minutes after exposure, neither of the response variables, $\%FEV_1$ and $\%A_P$, was correlated with either of the dosimetric variables, ozone uptake rate and ozone inhalation rate. Thus, the dependency of $\%A_P$ on ozone uptake rate disappeared between 10 and 70 minutes after exposure even though a finite negativity in $\%A_P$ persisted 70 minutes after (Table 6).

As a second stage in the uptake–response analysis, we performed a best-subsets regression analysis on $\%A_P$ and on $\%FEV_1$ measured at 10 minutes after exposure by introducing the following predictor variables in addition to ozone uptake rate: the dosimetric variable $VP_{50\%}$ (which characterizes the regional nature of ozone uptake); the anatomic variable dead space volume minus upper airways volume (which is related to the surface over which most ozone uptake occurs); and breathing frequency and tidal volume (which represent breathing pattern). Using the most favorable combination of these predictor variables, the R^2 value for the prediction of $\%FEV_1$ was only 0.055. Thus, $\%FEV_1$ appears to be unrelated to ozone uptake rate, $VP_{50\%}$, breathing frequency, tidal volume, and dead space volume minus upper airways volume. On the other hand, the R^2 value for the prediction of $\%A_P$ was improved from 0.149 to 0.189 when tidal volume was added as a predictor and to 0.310 when both breathing frequency and tidal volume were added. Thus, although we were unable to relate $\%FEV_1$ and corresponding dosimetric, anatomic, and breathing variables, $\%A_P$ could be represented in terms of ozone uptake rate and breathing pattern as:

$$\%A_P = -26.0 - 7.08 \text{ (ozone uptake rate [ppm-L/min])} + 0.943 \text{ (breathing frequency [bpm])} + 36.8 \text{ (tidal volume [L])}.$$

For this equation, $R^2 = 0.31$ and the (± SE, P) values for each estimated parameter were as follows: $\%A_P$ (16.7, 0.126), ozone uptake rate (1.83, 0.000), breathing frequency (0.355, 0.010), and tidal volume (11.9, 0.004). $\%A_P$ appeared to be unrelated to $VP_{50\%}$ and dead space volume minus upper airways volume.

Improved relations between decrements in response variables and ozone uptake were pursued by a number of additional approaches. For example, ozone uptake rate was normalized by a lung size parameter (dead space volume or FVC), absolute changes in FEV_1 and A_P were used in place of percent changes, and logarithmic transformations of ozone uptake rate, $\%FEV_1$, and A_P were implemented. None of these attempts were successful, however.

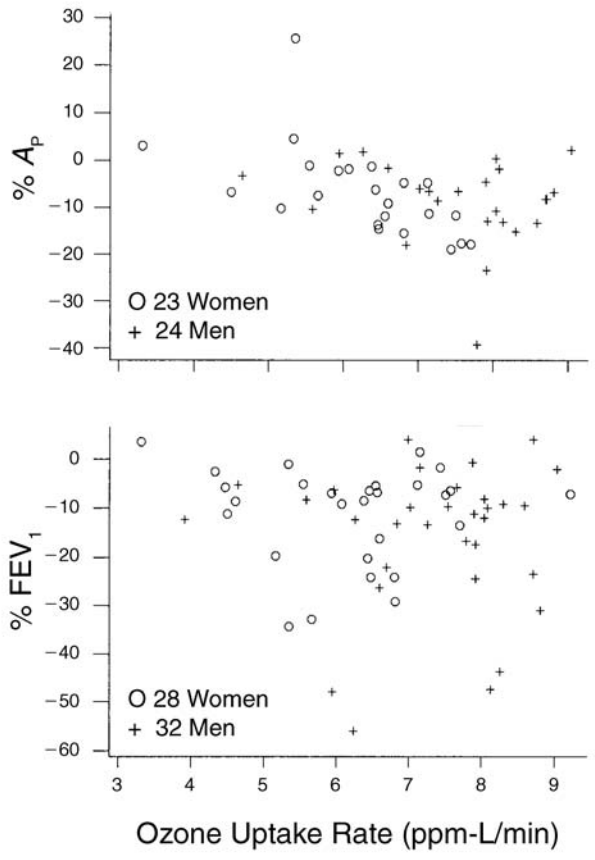

Figure 11. Individual responses and overall ozone dose during C_{O3} sessions. The correlation between $\%A_P$ and ozone uptake rate was significant (Pearson $r = -0.384$, $P = 0.008$); that between $\%FEV_1$ and ozone uptake rate was not (Pearson $r = -0.074$, $P = 0.575$). $\%A_P$ and $\%FEV_1$ were computed as 100[(10 minutes postexposure − preexposure)/preexposure].

DISCUSSION

The purpose of this research was to determine the influence of ozone uptake on intersubject differences in the acute response of the human respiratory system to ozone exposure. Because internal distribution of ozone, breathing pattern, and anatomy of airways and airspaces might all influence the transformation of overall ozone dose into a physiologic response, quantifying all three factors was an important aspect of this study. By and large, previous clinical studies of intersubject variation have ignored these factors and have tended to use inhaled ozone dose as a surrogate for actual ozone uptake. Moreover, measurements of physiologic response have been restricted primarily to decrements in plethysmographic variables (eg, airway resistance) or spirometric ones (eg, FEV_1). Such measurements primarily reflect changes in the conducting airways and are not strongly influenced by alterations within airspaces of the respiratory zone. In this study, we introduced A_P, a parameter derived from the phase III slope of the CO_2 expirogram (Figure 1), to better

characterize ozone-induced changes in the deepest regions of the lungs.

The concept of A_P has not been adopted by other investigators since it was first introduced by Scherer and associates (1983). Nevertheless, this parameter is very useful because it assigns anatomic meaning to the phase III slope, which has been the subject of much research since it was first discussed by Fowler (1948) more than 50 years ago.

In a study that combined CO_2 measurements and detailed numeric simulations of CO_2 diffusion, Schwardt and colleagues (1994) demonstrated that the steeper phase III slope exhibited by patients with chronic obstructive lung disease compared with normal subjects could be explained by a smaller cross-sectional area in the lung periphery. By simultaneously using mathematical modeling and CO_2 measurements, Huang and associates (2000) showed that as tidal volume increases, phase III slope is increasingly affected by the systematic accumulation of CO_2 in the respiratory zone. Yet another factor that affects phase III slope is ventilation inhomogeneity, which does in fact increase as a consequence of ozone exposure (Foster et al 1997). We concluded that the value of A_P computed from equation A.2 should be viewed as an effective peripheral cross-section that may be influenced by ventilation inhomogeneity and CO_2 accumulation in addition to diffusion limitations.

Because A_P is not a conventional parameter, published data with which to compare our data are limited. On the basis of the alveolar slope and CO_2 excretion rate of healthy subjects, Scherer and associates (1983) predicted that the value of A_P should be about 2000 cm^2, considerably less than the A_P (mean ± SD) of 3598 ± 75 cm^2 computed in our study. This difference is probably due to the modification of the calculation of A_P that we adopted to improve precision (Appendix A). This modification also fortuitously resulted in A_P values that better approximate the 5000 cm^2 that was estimated by Scherer from direct anatomic measurements. Our values of A_P are further supported by their correlation with the size of the respiratory system (represented by FVC; Figure 4, top panel).

Our study also differed from previous ones because of (1) the targeting of a constant minute ventilation; (2) the use of continuous exercise; and (3) the monitoring of ozone concentration and respired flow at the airway opening (the mouth only). Most investigators adjust workload in order to target a minute ventilation that is normalized by some measure of subject size such as FVC or $\dot{V}O_2max$. By adjusting workload to achieve the same minute ventilation for each subject irrespective of size, we intended to accentuate intersubject differences in breathing frequency and tidal volume.

In most previous studies, exercise was alternated with rest. The use of continuous exercise in this study resulted in a more straightforward, less ambiguous quantification of uptake.

In the majority of previous studies, subjects were exposed in a chamber containing a regulated ozone concentration and ozone inhalation rate was computed from the product of the measured minute ventilation and the ambient ozone concentration. Subjects in our study were exposed through a mouth-only breathing mask so that respired ozone concentration and flow could be continuously measured. Breathing patterns may be more natural in chamber exposures, but our ability to compute uptaken ozone breath by breath was a distinct improvement over previous work.

The work of Weinmann and associates (1995) is typical of previous studies of intersubject variation. Sixty-four healthy male nonsmokers were exposed in a chamber to 0.35 ppm ozone during two 30-minute intervals of treadmill exercise at a minute ventilation of 53 L/min alternated with 30-minute intervals of rest. Mean $\%FEV_1$ values were −16% immediately after exposure, −14% at 10 minutes after, and −12% at 30 minutes after. These data were consistent with the mean $\%FEV_1$ values for the 32 men in our study who exercised continuously but at a somewhat lower minute ventilation than Weinmann's subjects: −16% at 10 minutes and −12% at 70 minutes after exposure.

Weinmann also reported progressive increases in breathing frequency and decreases in tidal volume during the first exercise interval that were about 3 bpm and 0.2 L greater with ozone exposure than with air exposure, respectively. These changes are similar to the ozone-induced changes in breathing pattern observed in our study (Figure 6). What Weinmann did not investigate was the relation between physiologic response variables and the ozone uptake of each subject. In this respect, the studies of McDonnell and coworkers (1997) and Gerrity and coworkers (1994) were more comprehensive.

McDonnell and associates (1997) exposed 485 healthy young men to concentrations of 0 to 0.4 ppm ozone during 15-minute periods of treadmill exercise, which elicited minute ventilation values of 10, 29, or 37 L/min, alternated with 15 minutes of rest. Mathematical models of $\%FEV_1$ after 1 and 2 hours of exposure showed that [ambient ozone concentration × (minute ventilation)$^{0.9}$] (essentially the ozone inhalation rate) is a relevant predictor variable. Yet observed values plotted against predicted values of $\%FEV_1$ seemed to be largely uncorrelated. For example, at a predicted $\%FEV_1$ of −14%, observed $\%FEV_1$ varied fairly uniformly from 0% to −30%. This result is consistent with

the present finding that $\%FEV_1$ is not correlated with ozone inhalation rate.

Gerrity and coworkers (1994) are the only investigators who have attempted to correlate physiologic responses with uptake as well as inhaled dose (Gerrity et al 1994). Twenty healthy men were exposed to an ambient concentration of 0.4 ppm ozone for 1 hour during continuous treadmill exercise that elicited a mean minute ventilation of 41 L/min. Pre-to-postexposure changes in forced spirometry (eg, change in FEV_1 between end and start of exposure [ΔFEV_1]), plethysmography, and breathing pattern were measured as responses. The combined lower airway and airspace ozone uptake was measured by continuous monitoring of ozone in pharyngeal gas samples, but only for 5 minutes immediately after the beginning of exposure and for 5 minutes before the end of exposure. The current study employed an improved methodology that allowed ozone uptake to be measured throughout the exposure period.

Gerrity and colleagues reported that intersubject variation in ΔFEV_1 was not related to intersubject differences in lower airway ozone uptake. This result is consistent with our finding that $\%FEV_1$ was not related to $V_{P50\%}$, a parameter that characterizes distribution of the ozone uptake. Gerrity and coworkers also reported that ΔFEV_1 was linearly related to minute ventilation ($P = 0.011$). Given a constant ambient ozone concentration and exposure time, this finding indicates that ΔFEV_1 was linearly related to ozone inhalation rate as well. In contrast, we did not find $\%FEV_1$ and mean ozone inhalation rate to be correlated in our larger group of subjects. Our use of $\%FEV_1$ compared with Gerrity's use of ΔFEV_1 could have had the effect of normalizing size differences among individuals. We therefore computed ΔFEV_1 from our preexposure and postexposure values of FEV_1 but still found no correlation with mean ozone inhalation rate.

Perhaps the most important observation in our study was the difference in uptake response between FEV_1 and A_P. Values of these variables dropped dramatically during the 1-hour ozone exposure (C_{O3}) but not the 1-hour air exposure (C_a) (Table 6). Attempting to relate these responses to ozone uptake, we found that $\%FEV_1$ at 10 minutes after exposure was not correlated with mean ozone uptake rate. On the other hand, $\%A_P$ at 10 minutes after exposure was related to mean ozone uptake rate (Figure 11) as well as to breathing frequency and tidal volume. About 30% of the intersubject variation in $\%A_P$ was explained by the combination of these three variables.

Our ozone bolus distribution curves demonstrated that the lower conducting airways took up the majority of the ozone, but roughly 25% of the inhaled ozone was taken up in the respiratory zone (Figure 2). Therefore, the uptake-related processes contributing to $\%FEV_1$ may be saturated at the relatively large amounts taken up by the lower conducting airways whereas the processes responsible for $\%A_P$ remain responsive to the reduced uptake in the peripheral airspaces. An alternate explanation for our findings is that a background of intersubject variation in other factors (such as antioxidant status, C-fiber density, and upregulation of inflammatory mediators) affects FEV_1 more than A_P.

We believe that differences between ozone-induced decrements of FEV_1 and A_P were inevitable because the mechanisms underlying these responses are so different. FEV_1 is governed primarily by chest wall dynamics and airway mechanics, and ozone-induced decrements in FEV_1 originate in the conducting airways by a combination of smooth muscle contraction (Jones et al 1988; Murlas et al 1990) and decreased FVC caused by airway irritation (Hazucha et al 1989; Weinmann et al 1995). A_P, on the other hand, is closely related to the normalized phase III slope of a CO_2 expirogram (Appendix A, equation A.3), a well-established marker of pathophysiologic changes in the peripheral lung. For example, the phase III slope of a single-breath nitrogen washout was greater for smokers than for nonsmokers (Buist and Ross 1973) and was also proportional to the degree of small airway obstruction observed in excised lungs (Petty et al 1980).

Ozone diffusion through the epithelial lining fluid is attenuated by chemical reaction with antioxidants such as uric acid and vitamin C (van der Vliet et al 1999). This process prevents some of the taken up ozone from reaching underlying epithelium. According to simulations that account for local reaction rate and thickness of epithelial lining fluid (Miller et al 1985), the majority of the ozone taken up in the peripheral lung penetrates the epithelial surface, but only a small fraction of the ozone taken up in proximal airways actually reaches epithelium. Decrements in A_P are caused in large part by narrowing of airways or airspaces in the peripheral lung, whereas FEV_1 is mediated by receptors located in large airways. Therefore, our finding that the A_P response was related to mean ozone uptake rate but the FEV_1 response was not is logical. Furthermore, ozone-induced decrements in FEV_1 are directly affected by vitamin C ingestion (Samet et al 2001). Thus, natural variation in antioxidant status among subjects might induce corresponding variation in FEV_1 that obscures the effect of variation in overall ozone uptake.

Another interesting finding in this study was the lack of correlation between the responses of exercising subjects to ozone and air exposures (Figure 9). This result suggests that subtracting the response to air from the response to

ozone would not improve the precision of the data. This situation may explain why other investigators (eg, McDonnell et al 1983) have reported responses as pre-to-postexposure decrements in parameters of pulmonary function without referring to air controls. We also found that breathing pattern had an important effect on fractional ozone uptake efficiency. Uptake efficiency was inversely correlated with breathing frequency and directly correlated with tidal volume (Figure 7). Increased breathing frequency implies less time during each breath for ozone uptake, which explains why uptake would be less efficient and the A_P decrement would be reduced. On the other hand, increased tidal volume would drive inhaled ozone deeper into the lungs, thereby increasing uptake efficiency.

A disappointing result of this study was that intersubject differences in anatomic volume and internal ozone distribution did not account for intersubject variation in $\%FEV_1$ or $\%A_P$. We were able to show, however, that the $V_{P_{50\%}}$ in excess of the upper airways volume was linearly related to the lower airways volume (Figure 8). This new finding indicates that ozone is distributed to a greater cumulative airway volume within the respiratory system of a large individual than of a small individual. When viewed in terms of anatomic characteristics such as generation number, however, it may mean that ozone distribution is similar among different individuals irrespective of their airway volumes. Given the latter interpretation, it is not surprising that $\%FEV_1$ and $\%A_P$ were not related to $V_{P_{50\%}}$ and dead space volume.

SUMMARY AND CONCLUSIONS

Our continuous inhalation study of ozone was the first to measure dosimetric and ventilatory parameters throughout 1 hour of exposure. Important new findings in the dosimetric component of this study were that women had a significantly smaller mean uptake efficiency than men and that intersubject differences in uptake efficiency were inversely correlated with breathing frequency, directly correlated with tidal volume, and inversely related to duration of ozone exposure. At the relatively high minute ventilation of 30 L/min used in this study, very little ozone was taken up in the upper airways. Substantial uptake of an inhaled ozone bolus did occur, however, in both the lower conducting airways and the peripheral airspaces. $V_{P_{50\%}}$ was significantly smaller for the women than for the men.

In the physiologic response component of the study, continuous inhalation of ozone induced $\%FEV_1$ values from +4% to −56% and $\%A_P$ values from +26% to −39%. Mean $\%FEV_1$ (−13.7%) and mean $\%A_P$ (−7.80%) during the C_{O3} session were significantly ($P < 0.03$) lower than the corresponding mean values ($\%FEV_1$, −0.46%; $\%A_P$, +0.39%) during the C_a session. Although minute ventilation did not change systematically during exposures, inhalation of ozone caused a small but significant increase in breathing frequency and decrease in tidal volume as exposure proceeded. This result probably explains the slight but significant drop in uptake efficiency that also occurred during ozone exposure.

In the uptake–response component of the study, the mean decrements in FEV_1 and in A_P that occurred during ozone exposure were greater in men than in women, corresponding to the higher overall ozone uptake that the men received. Because $\%FEV_1$ was not correlated with mean ozone uptake rate, we conclude that overall ozone uptake is a poor predictor of intersubject variation in the response of conducting airways. On the other hand, because $\%A_P$ was reasonably correlated with mean ozone uptake rate, we conclude that overall ozone uptake is a predictor of intersubject variation in the response of distal airspaces. Intersubject variation in physiologic response that remained unexplained by uptake might be explained by interindividual differences in mucus composition, irritant receptor density, or upregulation of inflammatory mediators.

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APPENDIX A. Computation of A_P

The summed A_P was computed by a modification of the method originally suggested by Scherer and associates (1983). Longitudinal diffusion is governed by the Fick first law, which in the peripheral lung (where transport by bulk flow can be neglected) is given by

$$V_{CO_2} = -D_{CO_2} \times A_P \times (dC_{CO_2}/dz) , \qquad (A.1)$$

where V_{CO_2} is the volumetric rate of CO_2 elimination from the peripheral lung during exhalation, D_{CO_2} is the molecular diffusion coefficient of CO_2 in air (16 cm^2/sec), C_{CO_2} is the concentration of CO_2 expired from the peripheral lung, and z is longitudinal position in the lung. On the CO_2 expirogram, variation in CO_2 concentration in the peripheral lung can be estimated as the variation occurring in phase III (Figure 1). In that case, V_{CO_2} is the ratio of the area under phase III (AREA) to the time ($T_2 - T_1$) during which phase III occurs, and ($-dC_{CO_2}/dz$) is taken to be the product of the SLOPE of phase III and A_P. Substituting this information into equation A.1 and solving for A_P results in the following equation, which we used to compute A_P in this study:

$$A_P = \{\text{AREA}/[D_{CO_2} \times \text{SLOPE} \times (T_2 - T_1)]\}^{1/2} . \qquad (A.2)$$

An alternative computation of A_P can be obtained by recognizing that the ratio of AREA to ($T_2 - T_1$) is equivalent to the product of the mean expiratory flow (FLOW) and the mean expiratory concentration (CONC) during the phase

III portion of the expirogram. In that case, equation A.2 can reinterpreted as follows:

$$A_P = [(\text{FLOW}/D_{CO_2}) \times (\text{CONC}/\text{SLOPE})]^{1/2} . \qquad (A.3)$$

Because D_{CO_2} is a constant and FLOW was controlled at 250 mL/sec in this study, equation A.3 demonstrates that A_P should be inversely related to a normalized slope (SLOPE/CONC) of the alveolar plateau. Many previous studies of phase III have, in fact, reported their results in terms of normalized slope.

Equation A.2 differs from that of Scherer and associates (1983) in that V_{CO_2} was approximated by the expiration rate of CO_2 during phase III rather than as the mean expiration rate of CO_2 during the entire breath. This modification was intended to avoid any variation in A_P generated by variation in inhalation time immediately before measurement of the expirogram. As a result, the values for A_P computed from our procedure should be somewhat larger than those of Scherer and colleagues.

ABOUT THE AUTHORS

James S Ultman is distinguished professor of chemical engineering and bioengineering and chair of the Intercollege Graduate Degree Program in Physiology at the Pennsylvania State University. Dr Ultman has numerous publications in the areas of bioheat and biomass transfer including more than 30 articles regarding measurement and modeling of air pollutant uptake in the human respiratory system. Dr Ultman held sabbatical appointments as associate professor of chemical engineering at the Technion (Israel Institute of Technology) from 1977 to 1978 and research professor of medicine at Duke University Medical Center from 1989 to 1990. He received his PhD in chemical engineering from the University of Delaware in 1969 and was a National Institutes of Health postdoctoral fellow at the University of Minnesota from 1969 to 1970. Dr Ultman is a fellow of the American Institutes of Chemical Engineering and the American Institute of Medical and Biological Engineering.

Abdellaziz Ben-Jebria is professor of chemical engineering and a faculty member of the Bioengineering and Physiology Graduate Programs at Pennsylvania State University. Dr Ben-Jebria was a research scientist at the Institut National de la Santé et de la Recherche Médicale (INSERM, France) from 1980 to 1992. He received his PhD in biophysics and his state doctorate of science in natural sciences from the University of Pierre & Marie Curie (Paris, France) in 1979 and 1984, respectively. Dr Ben-Jebria is coinventor of three patents on porous particles for pulmonary drug delivery. He has authored or coauthored more than 65 publications in the area of lung dosimetry and respiratory toxicology of various inhalable materials.

Steven F Arnold is professor of statistics at Pennsylvania State University. Dr Arnold has published more than 30 theoretical papers in refereed journals and has authored or coauthored three graduate-level books in theoretical statistics, most recently the sixth edition of *Kendall's Advanced Theory of Statistics*. While at Penn State he has consulted with the Center for Locomotion Studies, the Pennsylvania Transportation Institute, the Applied Research Laboratory, the General Clinical Research Center, and the Colleges of Medicine, Science, Engineering, and Health and Human Development. He received his PhD in statistics from Stanford University in 1970.

OTHER PUBLICATIONS RESULTING FROM THIS RESEARCH

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Liu C. 2000. Statistical Analysis of Intersubject Variability in Pulmonary Response to Ozone Exposure [master's thesis]. Pennsylvania State University, University Park PA.

ABBREVIATIONS AND OTHER TERMS

$\%A_P$	percent change in A_P after exposure relative to start of exposure
$\%FEF_{25\%-75\%}$	percent change in $FEF_{25\%-75\%}$ after exposure relative to start of exposure
$\%FEV_1$	percent change in FEV_1 after exposure relative to start of exposure
%FVC	percent change in FVC after exposure relative to start of exposure
$\%\dot{V}O_2max@30$	$\dot{V}O_2$ as a percentage of $\dot{V}O_2max$ evaluated at a minute ventilation of 30 L/min
ΔFEV_1	change in FEV_1 between end and start of exposure
ANOVA	analysis of variance
A_P	cross-sectional area of the peripheral lung
B_{O3}	ozone bolus exposure
C_a	continuous air exposure
C_{O3}	continuous ozone exposure

CO_2	carbon dioxide
$FEF_{25\%–75\%}$	forced expiratory flow between 25% and 75% of FVC
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
Pearson *r*	Pearson product-moment correlation coefficient
R^2	coefficient of determination for multivariate analyses
$\dot{V}O_2$	oxygen consumption per unit time
$\dot{V}O_2max$	maximum oxygen consumption per unit time
$VP_{50\%}$	penetration volume at which 50% of an ozone bolus is taken up

CRITIQUE
Health Review Committee

Ozone is an irritant gas and a major component of smog. Some people exposed to ozone while undergoing moderate to strenuous physical activity experience reversible adverse responses in the lung (eg, irritation, inflammation, and decreased function). The degree of decreased lung function varies substantially among individuals exposed to the same level of ozone. Regulators need to know how ozone uptake in the respiratory tract is related to the subsequent biological responses in order to estimate the health risks of ozone exposure.

In addition to Requests for Applications (RFAs*), which target specific research areas, HEI issues Requests for Preliminary Applications (RFPAs), which invite short preliminary applications from investigators with interests that are outside those targeted in RFAs but are compatible with HEI's mission. In response to RFPA 97-3, "Request for Preliminary Applications on the Health Effects of Exposure to Air Pollutants from Motor Vehicle Emissions," Dr James Ultman of Pennsylvania State University submitted a preliminary application entitled "Distribution of Ozone in Intact Lungs: Intersubject Variability in Pulmonary Responses." Dr Ultman's primary hypothesis was that physiologic responses to ozone vary among people due to differences in the amount of ozone that actually reaches target tissues in the respiratory tract. His second hypothesis was that variation in breathing pattern and lung anatomy among people is responsible for these differences in ozone uptake by the respiratory tract. He proposed to test these hypotheses in healthy volunteers.

HEI has funded three studies by Dr Ultman (Ultman and Ben-Jebria 1991; Ultman et al 1994, 1997). The first two were to develop an ozone analyzer; the third was to improve its sensitivity and response time to rapid measurements of ozone uptake so that it could measure low levels of ozone in humans exercising at a moderate level. After reviewing the third study, the HEI Health Review Committee concluded that the ozone analyzer was able to quantitatively measure respiratory ozone uptake in exercising people. The Committee noted that regulators need to know how ozone exposure, dose to the respiratory tract, and subsequent biological responses are interrelated in order to estimate the risk that ozone exposure may pose to humans. They thought the ozone analyzer would be useful in addressing this need because dose estimated on the basis of exposure parameters and ozone concentrations ignores ozone absorption of the upper respiratory tract and does not account for differences among individuals. The current study followed through on the Review Committee's anticipated use of the ozone analyzer.

After reviewing Dr Ultman's preliminary application, the HEI Health Research Committee requested a full application that identified (1) an exercise and ozone exposure protocol that was appropriate for the proposed measurements and that ensured subject safety and (2) a study population that was sufficiently random to examine factors such as ethnicity, gender, and race. The Committee also asked Dr Ultman to enlist a statistician to develop a power calculation and a detailed protocol for data analysis. After Dr Ultman submitted a full application and addressed their concerns, the Committee recommended that his study be funded.†

SCIENTIFIC BACKGROUND

Individuals exposed to ozone by inhalation during moderate exercise vary in their respiratory response (as measured by decrements in spirometric measurements such as FEV_1 [forced expiratory volume in 1 second]). Some individuals show no change in FEV_1; others experience varying decreases that soon return to previous levels. In a key study, McDonnell and coworkers (1983) reported that men exposed to 0 to 0.4 ppm ozone for 2.5 hours showed decreases in FEV_1 that correlated with increases in ozone levels. However, the degree of decrement in FEV_1 at each ozone concentration varied greatly among the study subjects.

Wiester and colleagues (1996) found large variation in total ozone uptake in the respiratory tract (51% to 96%) in males at rest breathing 0.3 ppm ozone for 10 minutes. Rigas and collaborators (2000) found a much smaller range in ozone uptake (80% to 91%) in males breathing 0.2 or 0.4 ppm ozone for 60 minutes while exercising. Despite their differences, the results of these two studies indicate that males exposed to similar ozone levels take up different amounts in their respiratory system. (Similar studies have not been conducted with women.)

* A list of abbreviations and other terms appears at the end of the Investigators' Report.

† Dr Ultman's 3-year study, "Distribution of Ozone in Intact Lungs: Intersubject Variability in Pulmonary Response", began in January 1999. Total expenditures were $442,900. The draft Investigators' Report from Ultman and colleagues was received for review in April 2002. A revised report, received in March 2003, was accepted for publication in May 2003. During the review process, the HEI Health Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in both the Investigators' Report and in the Review Committee's Critique.

Ultman, Hu, and coworkers have characterized ozone uptake in different parts of the lungs by exposing male nonsmokers to a rapid dose of ozone (a bolus exposure with a peak of 4 ppm ozone) at various points during the inhalation phases of several quiet breaths (Hu et al 1992). They observed uptake efficiencies of 60% in the upper airways and virtually 100% in the upper and lower airways combined, with no uptake in deeper respiratory airspaces. When Hu and coworkers (1994) increased the airflow to a level reproducing air intake during exercise, the ozone uptake efficiencies fell to approximately 10% in the upper airways and 90% in the conducting airways and some ozone reached the lower respiratory airspaces. Thus, a distal migration of ozone was caused by increased ventilation. Bush and coworkers (1996) reported that men take up ozone more distally than women, probably because men have larger lung anatomic dead space (the volume of an inspired breath not involved in oxygen and carbon dioxide exchange) than women. These results suggest that individuals exposed to the same level of ozone may take up different amounts of ozone in the lungs because of differences in breathing pattern (at rest vs during exercise) and in lung anatomy.

Age may also contribute to variation in respiratory response to ozone. Drechsler-Parks and coworkers (1987) measured FEV_1 decrements in men and women undergoing intermittent moderate exercise after exposure to 0.45 ppm ozone for 2 hours. The older group (51–76 years of age) manifested smaller FEV_1 decrements than the younger group (18–26 years), an observation also noted by McDonnell and coworkers (1995).

Variation in respiratory response to ozone may also be affected by gender. For example, Lauritzen and Adams (1985) noted that the correlation between FEV_1 decrements and ozone levels (0 to 0.4 ppm for 1 hour with continuous exercise) was stronger in women 22 to 29 years of age than in men. However, male lungs are generally larger than female and additional measurements suggested that the different responses might be due to variation in ozone uptake that is inversely related to lung size. In contrast, Adams and colleagues (1987) found no difference in FEV_1 decrements between men and women exposed to 0.3 ppm ozone during 1 hour of continuous exercise. Seal and coworkers (1993) observed that FEV_1 decrements in African-American and white men and women exposed to 0 to 0.4 ppm ozone for 2 hours during intermittent exercise correlated with ozone levels, but the results did not differ significantly among the study groups (grouped by race and sex). The design of the study ensured that each subject received the same ozone dose per unit lung volume at each concentration. Therefore, changing the ventilation rate (and therefore the uptake of ozone) may affect intersubject variation in ozone-induced FEV_1 decrements.

The studies described above indicate that intersubject variation in respiratory response to ozone may depend on many factors. The current study by Ultman and colleagues addressed the hypotheses that (1) variation in individual responsiveness to controlled ozone exposures is related to individual differences in ozone uptake and (2) much of the individual difference in ozone dose that induces this variation is caused by intersubject variation in breathing patterns and airway anatomy.

TECHNICAL EVALUATION

SPECIFIC AIMS

The specific aims of the study were to determine:

1. The relation of lung anatomy and breathing pattern to overall uptake of ozone;
2. The relation of lung anatomy to the distribution of ozone within the lung (referred to as the *regional distribution* of ozone);
3. The relation between overall ozone uptake and physiologic responses; and
4. The relation between indicators of regional ozone uptake and response parameters.

STUDY DESIGN

Thirty-two men and twenty-eight women who were nonsmokers completed the study. They underwent a health screening session and then three types of exposure sessions: bolus ozone inhalation, continuous air inhalation, and continuous ozone inhalation. Each type of exposure session was performed in the order listed with at least 2 weeks between types.

The authors suggest that this exposure sequence ensured no carryover effect of ozone on the air exposures. In addition, performing the continuous ozone exposure last may have maximized retention among the most ozone-sensitive subjects who might otherwise have terminated their participation. However, the nonrandom exposure sequence raises the possibility of a sequence effect on the measurements independent of an ozone-specific effect.

Bolus Ozone Inhalation to Assess Distribution of Ozone Uptake

In the first type of exposure, the investigators measured the regional distribution of ozone uptake within the respiratory

tract by bolus inhalation, a noninvasive procedure. Each session consisted of the collection of a series of separate bolus test breaths. During each test breath, the subject grasped the mouthpiece of a breathing assembly and matched his or her breathing rate to a respired volume pattern displayed on a monitor. For each consecutive test breath, 20 mL of an air–ozone mixture (the bolus) was injected into the inhaled airstream earlier and earlier relative to the end of inspiration. (The peak ozone concentration of the bolus was ~1 ppm.) In this way, the uptake of ozone was measured deeper and deeper within the airways. The investigators continuously recorded respired flow and ozone concentrations at the subject's lips. Ozone concentration was monitored using the ozone analyzer they had previously developed (Ultman and Ben-Jebria 1991; Ultman et al 1994, 1997), which reliably measures ozone levels during the conditions of rapid respiration in this study. The bolus exposure was terminated after 60 to 80 test breaths had been taken.

Continuous Air and Continuous Ozone Exposures

In the continuous air inhalation and continuous ozone inhalation protocols, subjects breathed clean air or air containing a mean concentration of 0.252 ppm ozone (0.3 ppm target concentration), respectively, through a mouth-only breathing mask for 1 hour. Each exposure was conducted under conditions of moderate exercise.

METHODS

- During bolus ozone exposures, two parameters were measured for each test breath: the uptake fraction of ozone (the net amount of ozone taken up relative to the inhaled amount) and the penetration volume (V_P; the volume of air inhaled between introduction of the ozone bolus and the end of inspiration). Penetration volume approximates the cumulative airways volume reached by the bolus during inhalation; it can therefore be associated with specific anatomic landmarks when the corresponding anatomic volumes are known. The penetration volume data were expressed as $V_{P_{50\%}}$ (penetration volume at which 50% of an ozone bolus is taken up). The $V_{P_{50\%}}$ values (indicating the regional nature of ozone uptake) allowed the investigators to quantify intersubject differences in bolus uptake.
- In each of the three types of exposures, each subject's anatomy was characterized by acoustic reflectometry (upper airways volume and upper airways length) and by carbon dioxide expirometry (total conducting airways volume). The volume of the lower airways was obtained by subtracting upper airways volume from anatomic dead space volume.
- Carbon dioxide expirometry was also used to measure cross-sectional area of the peripheral lung (A_P) during continuous air and continuous ozone exposures. The authors consider this measurement to be an index of parenchymal function or anatomy. A_P data were quantified as the percent change after exposure relative to start of exposure (%A_P).
- Before and 10 and 70 minutes after the continuous air or continuous ozone exposures, spirometry was used to measure pulmonary responses—FEV_1, forced vital capacity (FVC), and forced expiratory flow between 25% and 75% of FVC ($FEF_{25\%–75\%}$). Because all three variables correlated well, the investigators reported only the results of the FEV_1 measurements. FEV_1 responses were quantified as the percent change after exposure relative to start of exposure (%FEV_1).
- A_P, anatomic dead space volume, upper airways length, and upper airways volume were measured before exposures. A_P and dead space volume were also measured 10 and 70 minutes after continuous air and continuous ozone exposures.
- Average tidal volume (the volume of air expired with each breath) and average breathing frequency were determined during continuous air or continuous ozone exposures.
- Overall ozone uptake and uptake efficiency were determined during ozone exposure.

STATISTICAL ANALYSES

The investigators determined the contribution of day-to-day variation to total variance by performing each of the three exposure types twice with the first ten subjects, five men and five women. They combined the results of the first set of exposures for these first ten subjects with results of the single set of exposures performed on each of the remaining subjects. Ultman and coworkers were unable to collect all data prescribed for all research sessions on each subject. They considered a session to be complete if (1) a complete set of ozone bolus data was collected, (2) complete sets of continuous air and continuous ozone uptake data were recorded, and (3) FEV_1 was measured at the planned time points in the continuous air and continuous ozone exposures.

Ultman and colleagues employed four types of statistical analyses to analyze the anatomic, dosimetric, and physiologic response variables. They used Pearson correlations to determine the degree to which pairs of continuous variables were related and used best-subset regressions to simultaneously evaluate the influence of more than one continuous predictor variable. They used analysis of variance (ANOVA) to determine the significance of subject as a

random variable nested in sex, sex as a fixed variable, continuous ozone or continuous air exposures as fixed variables, and session replication as a fixed variable. Between-subject variation was determined as a percentage of total variation by a components of variance analysis (part of each ANOVA). In some cases, the investigators performed a series of *t* tests rather than a single ANOVA to improve interpretation of their data. In these analyses, they confirmed the equality of variance and normality with residual plots and considered data with *P* values less than 0.05 to be significant.

RESULTS AND DISCUSSION

This study was performed by experts in evaluating dosimetry of inhaled ozone in humans. The key results were as follows.

As expected, ozone exposure caused a wide range of decrements in FEV_1. Of 60 total subjects, 20 showed decrements greater than 15% of their preexposure baseline; four males showed decrements greater than 40%. This wide range suggests that the study tested an adequate number of subjects.

Values of $\%FEV_1$ and $\%A_P$ showed significant decrements at 10 and 70 minutes after ozone exposure. The authors hypothesized that if the area of ozone uptake influences airflow (A_P is a measure of the cross-sectional bronchial area of the peripheral lung), the two parameters should be correlated. However, there was no significant correlation between A_P and FEV_1 (a well-accepted method of measuring pulmonary function). This result suggests that these parameters represent independent changes in lung function after exposure to ozone, because they either measure fundamentally different processes or they interrogate different regions of the lung. Use of A_P was a novel approach, but it may be questionable because changes in A_P have not been validated as responses to ozone.

Ten minutes after ozone exposure, ozone uptake rate did not correlate with $\%FEV_1$ but did correlate with $\%A_P$. (Neither $\%FEV_1$ nor $\%A_P$ correlated with ozone uptake rate 70 minutes after exposure.) The finding that individual variation in FEV_1 was not related to differences in ozone uptake does not support the investigators' first hypothesis. The investigators also found no relation between $\%FEV_1$ and $V_{P50\%}$ (which characterizes the distribution of ozone uptake). These findings agree with those of Gerrity and coworkers (1994) that intersubject variation in preexposure to postexposure changes in FEV_1 is not related to differences in the ozone dose taken up by the lower airways.

In contrast, Ultman and colleagues suggest that the correlation between ozone uptake rate and $\%A_P$ could be interpreted as substantiating their first hypothesis with respect to intersubject variation in the distal airspaces. This interpretation is hard to accept, however, because the physiologic significance of changes in A_P has not been established. (See Scherer and colleagues [1983] for a discussion of factors that influence A_P.) In addition, few other studies have measured A_P, so comparing Ultman's values with those reported elsewhere is difficult.

Ultman and colleagues found that uptake efficiency of ozone was affected by airway anatomy. For example, uptake efficiency varied among subjects and this variation was significantly lower in women than in men. (On average, the men had larger lungs than the women.) Uptake efficiency was inversely correlated with breathing frequency and directly correlated with tidal volume. Increased breathing frequency allows less time for ozone uptake during each breath. Increased tidal volume drives inhaled ozone more deeply into the lungs, increasing the uptake efficiency. Thus, these data validated the investigators' second hypothesis that much of the variation in ozone dose is caused by intersubject variation in airway anatomy and breathing pattern. These findings are not new, but they are consistent with the results of previous studies in humans (Gerrity et al 1988), rats and guinea pigs (Wiester et al 1988), and dogs (Yokoyama and Frank 1972). They add to the usefulness of the Investigators' Report.

A new finding related to airway anatomy was that $V_{P50\%}$ increased with increasing lower airways volume. This finding is consistent with the inverse relation between surface-to-volume ratio and volume of the lower conducting airways. For example, the mean $V_{P50\%}$ for women in this study was significantly lower than the mean $V_{P50\%}$ for men. The larger surface-to-volume ratio characteristic of the smaller female airways increases ozone uptake in the upper conducting airways and reduces the penetration volume of ozone.

SUMMARY AND CONCLUSIONS

Ultman and colleagues investigated two hypotheses:

1. Variation in individual responsiveness to controlled ozone exposure relates to individual differences in ozone uptake, and
2. Much of the individual differences in ozone uptake that induce this variation is caused by intersubject variation in breathing patterns and airway anatomy.

Thirty-two men and twenty-eight women, all nonsmokers, completed the study. The investigators measured

the regional distribution of inhaled ozone by the bolus inhalation method. After 2 weeks, they exposed the subjects to clean air and 2 weeks later to 0.25 ppm ozone for 1 hour under conditions of moderate exercise. The investigators measured the length and volume of the upper airways before air or ozone exposure. They measured changes in FEV_1 and A_P before and after continuous air and ozone exposures and calculated the percent change after exposure relative to the start of exposure, $\%FEV_1$ and $\%A_P$. They also measured average breathing frequency during these exposures and measured inhaled ozone concentration, ozone uptake rate, and tidal volume during the ozone exposure.

As expected, FEV_1 decrements varied widely after ozone exposure. Ten minutes after ozone exposure, $\%FEV_1$ did not correlate with ozone uptake rate, indicating that individual variation in response to ozone as measured by FEV_1 cannot be explained by differences in ozone uptake. This result does not support the first hypothesis. In contrast, $\%A_P$ and ozone uptake rate were correlated; the investigators interpreted this finding as support for their first hypothesis. Although use of A_P was a novel approach to evaluating changes in the transport properties of the lungs, uncertainty regarding the physiologic significance of A_P to ozone exposure detracts from the strength of this interpretation.

The investigators' second hypothesis was validated. They found that ozone uptake efficiency was affected by airway anatomy and breathing pattern. Intersubject variation in uptake efficiency was significantly lower in women than in men. It also was inversely correlated with breathing frequency and directly correlated with tidal volume. Increased breathing frequency allows less time for ozone uptake during each breath; increasing tidal volume drives inhaled ozone more deeply into the lungs, increasing uptake efficiency. Ultman and colleagues' data are consistent with the results of earlier studies and add to the usefulness of the Investigators' Report. They also reported a new finding: increases in lower airways volume were associated with increases in one measure of ozone uptake.

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HEALTH
EFFECTS
INSTITUTE

Charlestown Navy Yard
120 Second Avenue
Boston MA 02129-4533 USA
+1-617-886-9330
www.healtheffects.org

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