Effect of C-fiber-mediated, ozone-induced rapid shallow breathing on airway epithelial injury in rats

E. S. SCHELEGLE, M. F. ALFARO, L. PUTNEY, M. STOVALL, N. TYLER, AND D. M. HYDE
Department of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, University of California, Davis, California 95616

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Schelegle, E. S., M. F. Alfaro, L. Putney, M. Stovall, N. Tyler, and D. M. Hyde. Effect of C-fiber-mediated, ozone-induced rapid shallow breathing on airway epithelial injury in rats. J Appl Physiol 91: 1611–1618, 2001.—We examined the relationship between C-fiber-mediated, ozone-induced rapid shallow breathing and airway epithelial cell injury at different airway sites within the lower respiratory tract of conscious Wistar rats (n = 24). We combined an acute 8-h ozone inhalation with vagal perineural capsaicin treatment, a selective C-fiber conduction block, and 5-bromo-2′-deoxuryridine (BrdU) labeling as an index of epithelial injury. Vehicle-treated rats that inhaled ozone developed a rapid shallow breathing pattern during ozone inhalation, whereas the capsaicin-treated rats that inhaled ozone showed no changes in respiratory frequency. In vehicle-treated, ozone-exposed rats that developed rapid shallow breathing, a progressive increase in BrdU-labeling density (no. of BrdU-labeled cells/mm² airway) was observed starting at the bifurcation of the left main stem bronchi (central airway) and going down either a short or long airway path. In vehicle-treated, ozone-exposed rats, terminal bronchioles supplied by short and long airways had a similar degree of BrdU-labeling density that was significantly (P < 0.05) greater than the BrdU-labeling density of the proximal airways that supply them. In contrast, the attenuation of rapid shallow breathing produced by capsaicin treatment resulted in a significantly reduced BrdU-labeling density in the terminal bronchioles supplied by short airway paths compared with the terminal bronchioles supplied by long airways. Our data indicate that ozone-induced rapid shallow breathing protects large conducting airways while producing a more even distribution of injury to terminal bronchioles.

THE ACUTE INHALATION OF OZONE, the primary oxidant in urban photochemical air pollution, has been shown to result in epithelial injury and inflammation at sites within the nasal cavity and throughout the tracheobronchial tree in animals (10, 12, 21, 28) and humans (13, 24). Theoretical models of ozone uptake predict that several factors may affect which airway region is injured by ozone. These factors include, but are not limited to, airway branching geometry, antioxidants such as uric acid and ascorbic acid in the airway lining fluid, ventilation, and breathing pattern (19, 20). Acute ozone inhalation has been shown to evoke numerous physiological responses in both animals and human subjects (1, 18, 30, 31) that are associated with this epithelial injury and inflammation. These physiological responses include rapid shallow breathing, bronchoconstriction, a decrease in inspiratory capacity, and respiratory symptoms of discomfort (1, 18, 30, 31). Of these physiological responses, it has been proposed that rapid shallow breathing is a protective response initiated by lung sensory C fibers (4, 8, 25) to limit the extent of injury to the distal respiratory tract (20, 29, 31).

Using a theoretical model designed to simulate ozone uptake in the tracheobronchial tree of rats, Overton et al. (20) examined whether the previously observed (3, 28) random pattern of epithelial injury induced by prolonged ozone inhalation within different centriacinar regions of rats is a function of airway path length and breathing pattern. Boorman et al. (3) speculated that this random centriacinar injury pattern was due to variations in path length and the number of intervening airway generations but did not consider the effect of changing breathing pattern. On the basis of their theoretical model, Overton et al. (20) predicted that, during the transition from eupnic breathing to rapid shallow breathing, the centriacinar regions supplied by long airway paths would receive progressively lower ozone concentrations compared with short airway paths. This decrease in ozone delivery to centriacinar regions supplied by long airway paths is the combined effect of greater volume and surface area of the longer airways paths and the decrease in tidal volume (V̇T) present during rapid shallow breathing.

In the present study we examined the relationship between C-fiber-mediated, ozone-induced rapid shallow breathing and airway epithelial cell injury at different regions within the lower respiratory tract. We accomplished this by combining an acute 8-h ozone inhalation with a selective vagal C-fiber conduction block and 5-bromo-2′-deoxuryridine (BrdU) labeling as an index of epithelial injury (23) in conscious rats. By sampling at sites along short and long airway paths,
we examined the interaction between reflex rapid shallow breathing and airway path length on the site-specific severity of ozone-induced injury.

**MATERIALS AND METHODS**

**Experimental protocol.** Twenty-four pathogen-free Wistar rats (Charles River Laboratories, Kingston, NY) were housed in a bioclean facility and assigned to four groups. These groups were as follows: vehicle treated that inhaled filtered air (vehicle/filtered air; \( n = 8 \)), vehicle treated that inhaled ozone (1 part/million) (vehicle/ozone; \( n = 7 \)), capsaicin treated that inhaled filtered air (capsaicin/filtered air; \( n = 6 \)), and capsaicin treated that inhaled ozone (1 part/million) (capsaicin/ozone; \( n = 7 \)). Twenty hours before ozone inhalation, rats were anesthetized intraperitoneally with a mixture (1.0 ml/kg) of ketamine (100 mg/ml) and xylazine (20 mg/ml). By using sterile surgical technique, a mid sagittal incision along the ventral side of the neck was performed to access both vagus nerves. The strap muscles were held apart with a pair of retractors, and, by using fine surgical instruments and blunt dissection, the carotid sheath was removed and the vagus nerve was freed from the carotid artery. To block the conduction of vagal afferent C fibers, Kimwipes pledgets soaked with 1.0% capsaicin in cold-pressed, extravirgin olive oil (G. Sensat, Linares, Spain) were placed topically on each nerve for 2–10 min. A strip of 1-ml plastic sheet (4.0 mm wide) was placed under one vagus nerve, over the trachea, and under the other vagus nerve to prevent capsaicin from coming into contact with the surrounding tissue. Vehicle (olive oil)-treated rats had pledgets soaked in olive oil placed on the nerve for 2 min. The pledgets were removed, and any excess capsaicin solution and/or olive oil was removed with a sterile cotton-tip applicator. The plastic strip was removed, and the incision was closed with 7.5-mm stainless steel wound clips (Roboz Surgical Instrument). A miniosmotic pump (ALZET pump model 2ML1, nominal pumping rate 10 \( \mu \)l/h; ALZA, Palo Alto, CA), primed and loaded with BrdU at 30.0 mg/ml (Sigma Chemical, St. Louis, MO) in 0.01 N NaOH, was then placed subcutaneously between the shoulder blades of the anesthetized rats 20 h before ozone inhalation. The pumps were primed by immersion in 0.9% saline at 37°C 8 h before insertion. The rats regained consciousness and were allowed to recover overnight from the surgical and experimental treatment. On the following day rats were placed one rat per chamber in 8-liter Pyrex glass chambers in which the whole mounts were incorporated into the chamber design: one inside the chamber itself on which the rat was placed and the other between the lid and the chamber compartment. A hole in the center of the chamber lid allowed air to flow into the top compartment, through the middle and bottom compartments, and to be exhausted from a low-reactance glass tube passing through the center of the chamber. All flows into the chamber were carried through Teflon tubing, and outflow traveled through the glass tube connected to a pneumotachograph (model 4700, Hans Rudolph, Kansas City, MO). Chamber temperature (°C) and relative humidity (RH, %) were measured between the chamber lid and the pneumotachograph using a temperature-RH probe (model 50Y, Vaisala, Irvine, CA). Chamber pressure was measured using a solid-state differential pressure transducer (SenSym, Milpitas, CA) via a second port in the lid. We generated ozone by passing oxygen through an ozonizer (model 1003-AH, Dasibi Environmental, Glendale, CA). The ozone analyzer was calibrated using the ultraviolet-absorption photometric method at the University of California, Davis, California Regional Primate Research Center.

**Measurement of breathing pattern.** Respiratory frequency (\( f \)) and an estimate of \( V_t \) in the unrestrained, conscious rats during the experimental protocols were obtained and averaged over each minute by monitoring the small variation in chamber pressure associated with heating and humidification of air during inspiration. An estimate of \( V_t \) was obtained using the equations of Drorbaugh and Fenn (5). These equations used the integral of the variation in chamber pressure, chamber temperature, and RH while assuming a constant body temperature for the rats of 36.5°C. An estimate of minute ventilation (\( V_e \)) was calculated post hoc by multiplying \( f \) and the estimate of \( V_t \). All data were collected and analyzed using a digital data acquisition system (BioSystem XA, Buxco Electronics, Sharon, CT).

**Necropsy, lung microdissection, and BrdU incorporation.** BrdU, a thymidine analog incorporated into DNA by cells undergoing DNA replication, is indicative of cell proliferation and therefore a marker of ozone-induced airway epithelial injury (23). The duodenum served as a positive labeling control for each rat. The incorporated BrdU was detected using monoclonal antibody and immunocytochemical techniques as outlined by Hsu and co-workers (9). Rats were anesthetized intraperitoneally with 4% pentobarbital sodium solution, and the trachea was cannulated. The abdomen was opened, and cutting the descending aorta exsanguinated the rat. The chest was opened, and the lungs were fixed by intratracheal instillation with a buffered formalin fixative (Z-Fix, Anatech, Battle Creek, MI) at a pressure of 30 cmH2O. Lungs and duodenums were then stored in the same fixative for at least 48 h before processing. Beginning at the level of the lobar bronchus, we used a dissecting microscope and a cool fiber-optics illuminator to microdissect the fixed left lung lobe along its long axis (Fig. 2A). This dissection plane through the lobe exposed the majority of small side branches. Whole mounts of the microdissected left lung lobe were incubated in 2.5 N HCl for 30 min at 37°C to denature the DNA. The acid was neutralized with two borate buffer washes (5 min each) and a PBS wash (5 min). The whole mounts were incubated in 3% hydrogen peroxide for 30 min to block the endogenous peroxidase activity, washed for 10 min in PBS, and then incubated in 5% normal rabbit serum with 5% powdered milk for 1 h to block nonspecific binding. After excess fluid was blotted, the whole mounts were incubated with a primary mouse anti-BrdU (Dako, Carpinteria, CA) diluted 1:50 in PBS with 5% powdered milk for 2 h. After being washed with PBS, the whole mounts were incubated through the glass tube connected to a pneumotachograph (model 4700, Hans Rudolph, Kansas City, MO). Chamber temperature (°C) and relative humidity (RH, %) were measured between the chamber lid and the pneumotachograph using a temperature-RH probe (model 50Y, Vaisala, Irvine, CA). Chamber pressure was measured using a solid-state differential pressure transducer (SenSym, Milpitas, CA) via a second port in the lid. We generated ozone by passing oxygen through an ozonizer (model 1003-AH, Dasibi Environmental, Glendale, CA). The ozone analyzer was calibrated using the ultraviolet-absorption photometric method at the University of California, Davis, California Regional Primate Research Center.
for 60 min in biotinylated, rabbit anti-mouse immunoglobulin (Dako) diluted 1:200 in PBS with 5% normal rabbit serum. This procedure was followed with two PBS washes and incubation of the sections in Vectastain ABC reagent (Vector Laboratories, Burlingame, CA) for 30 min. After two washes in PBS (5 min each), the whole mounts were incubated in peroxidase substrate solution (7.5 μl 30% hydrogen peroxide in 1.0 mg/ml diaminobenzidine in 0.1 M Tris·HCl buffer at pH 7.6) for 3–5 min. Finally, the whole mounts were rinsed with PBS and then distilled water. Labeled nuclei appeared reddish-brown using a dissecting microscope, and no counterstain was used.

**Estimation of cell proliferation.** Using a stereomicroscope at ×150, the number of BrdU-positive cells was counted within the visual field at five sites along the airway microdissections (Fig. 2B). These sites included a central airway (CA) site and four accompanying sites [proximal along a short and long airway path (SPPA and LPPA, respectively) and terminal bronchioles supplied by these short and long airway paths (SPTB and LPTB, respectively)]. The counting required focusing over several hundred micrometers to include the walls and the base of the selected airway location. Counts were expressed as number per average surface area of counting region (no./mm²). This was done by measuring heights, widths, and angles in all of the airway sites in three rats followed by trigonometric calculations of the appropriate surface area. For terminal bronchioles and other curved airways, we used the formula for the curved surface of a cylinder (\( A = 2\pi r l \)). Because we were evaluating airways cut in half longitudinally, we modified the formula to \( A = \pi rl \), where \( r \) is the measured radius of the airway, \( l \) is the length the airway in the field of view, and \( \pi = 3.14159 \). For the most proximal airway, CA, where the cells were counted on the ridge of a bifurcation, we measured the height of the bifurcation using a length gauge on a stereomicroscope and the base width of the bifurcation from one epithelial surface to the next. Using the Pythagorean theorem of \( C^2 = A^2 + B^2 \), we determined the true epithelial length from the bifurcation apex to its base. This distance was then multiplied by the length of the bifurcation in the field of view to determine the area of one side. This process was repeated for the other side and the results added to give the total surface area of CA.
Statistical analysis. All data were expressed as means ± SE. Thirty-minute running averages were computed using the raw minute averages collected during the experimental time sequence for $f$, $V_T$, and $V˙E$, with the first value calculated from the control period data. From these 30-min averages, we selected the value at consecutive 30-min time points over the remainder of the 12-h experimental time sequence and expressed as percentage of control. This breathing pattern data were analyzed using a three-way analysis of variance with repeated measures using two grouping factors (exposure condition and vagal treatment) and within factor (time of exposure) (Statview, SAS Institute, Cary, NC). BrdU labeling was expressed as the absolute number of BrdU-labeled cells contained within the microscope field and within the airway location being examined. BrdU-labeled cell counts were analyzed using one- or two-way analysis of variance. All significant mean differences were determined using Fisher’s protected least significant difference test (Statview, SAS Institute). Statistical significance was accepted for $P ≤ 0.05$.

RESULTS

Breathing pattern. Statistical analysis showed that there was no significance between the vehicle/filtered air-treated ($n = 8$) and the capsaicin/filtered air-treated ($n = 6$) groups for $f$, $V_T$, and $V˙E$, and as a result these two groups were combined. Vehicle/ozone-treated rats ($n = 7$) had a significant increase ($P ≤ 0.05$) in $f$ (50%) at 120 min, which leveled off at 210 min and remained elevated for the remainder of the data collection period (Fig. 3A). The value of $f$ was not affected in the capsaicin/ozone-treated rats ($n = 7$) other than a nonsignificant 20% increase at 330 min, which was sustained for the remainder of the data collection period (Fig. 3A). The vehicle/ozone group had a significant decrease ($P ≤ 0.05$) in $V_T$ that began at 90 min and continued through the end of the ozone inhalation period (Fig. 3B). A slight attenuation took place during postexposure, but values remained significantly decreased. The capsaicin/ozone group had a nonsignificant decrease in $V_T$ (25%) from 210 to 600 min that was attenuated in the 4-h postrecovery period (Fig. 3B). There were no significant effects of ozone inhalation on $V˙E$ in either vehicle- or capsaicin-treated rats (Fig. 3C).

BrdU-labeled cell counts. Figure 4 contains images collected using a stereomicroscope showing the distribution of BrdU-labeled cells in a proximal airway (Fig. 4). Statistical analysis showed that there was no significant differences between the vehicle/filtered air-treated ($n = 4$) and the capsaicin/filtered air-treated...
(n = 6) groups for BrdU-labeled cells at any airway site, and as a result these two groups were combined. In rats that inhaled filtered air, there was significantly greater (P < 0.05) BrdU labeling in the LPPA and SPPA sites compared with the CA site, demonstrating differences in baseline regional cell proliferation within the airways sampled (Fig. 5A).

Ozone inhalation in the vehicle-treated rats that developed rapid shallow breathing resulted in a progressive increase in BrdU labeling moving from the CA to the SPTBs and LPTBs (Fig. 5B). BrdU labeling in the SPTBs and LPTBs was significantly greater than the BrdU labeling in the CA. Compared with the rats that inhaled filtered air, there was significantly greater BrdU labeling at the CA, SPTB, and LPTB sites in the vehicle-treated rats that inhaled ozone.

In contrast, ozone inhalation in the capsaicin-treated rats, which showed a significant attenuation of ozone-induced rapid shallow breathing, resulted in an altered pattern of BrdU labeling compared with the vehicle-treated rats that inhaled ozone (Fig. 5C). Ozone inhalation in the capsaicin-treated rats resulted in a progressive increase in BrdU labeling from the CA to the LPTB only. The BrdU labeling was significantly greater in the LPTBs compared with the CA and SPTBs. When capsaicin-treated rats that inhaled ozone were compared with the rats that inhaled filtered air, there was significantly greater BrdU labeling in the CA and LPTB sites only. In addition, the BrdU labeling in the CA of the capsaicin-treated rats that inhaled ozone was significantly greater than that counted in the vehicle-treated rats that inhaled ozone.

DISCUSSION

The results of the present study definitively establish the role of lung vagal C fibers in rapid shallow breathing induced by acute inhalation of ozone in conscious rats. Furthermore, these results demonstrate the role that this reflex rapid shallow breathing plays in the severity of airway epithelial injury at different sites within the lower respiratory tract. The pattern of injury in the presence of rapid shallow breathing was such that the more proximal airways showed less injury and the SPTB and LPTB showed an even distribution of injury. In contrast, the attenuation of rapid shallow breathing resulted in an uneven distribution of injury to the terminal bronchioles studied, with significantly less injury being present in the SPTB compared with the LPTB. These data indicate that C-fiber-mediated rapid shallow breathing is protective to proximal bronchi while increasing the extent of injury to terminal bronchioles that are supplied by airway paths of varying length.

We used vagal perineural capsaicin treatment to block the conduction of vagal sensory C fibers in the present experiment for two reasons. First, several studies using similar concentrations of capsaicin in oil (1%) have shown perineural capsaicin treatment produces a selective conduction block of sensory C fibers in large, mixed nerve trunks in rats that lasts up to 4 mo (2, 14–17, 26). The use of the compound action potential to study perineural capsaicin treatment in our laboratory (17, 26) and in that of others (2) definitively establishes the selective nature of the conduction block produced. The results of the present experiment would
therefore indicate that increases in f induced by ozone inhalation and to a lesser extent decreases in VT are mediated by vagal sensory C fibers.

Second, unlike systemic capsaicin treatment, peripherally capsaicin treatment does not appear to produce a large depletion of neuropeptides in the peripheral site innervated by the treated nerve within the time frame in which the present study was conducted (6, 26). This point is important given the previous observations of Vesely et al. (31) that neonatal capsaicin treatment, which produces a chronic depletion of peripheral neuropeptides, significantly depressed the baseline epithelial cell proliferation. Our observation that BrdU labeling was not influenced by peripherally capsaicin treatment in the filtered air-exposed rats suggest that the depletion of peripheral neuropeptides did not significantly influence the results of these experiments. Therefore, BrdU incorporation into DNA during epithelial cell proliferation, as used in other studies, can be considered a valid, sensitive, and quantitative indicator of ozone-induced injury throughout the respiratory tract of rats, including the nasal cavity, large airways, and terminal bronchioles (11, 23, 32).

We did not attempt to identify the cell type that was proliferating in the present experiment and used BrdU labeling as index of cell proliferation after ozone-induced injury only. Because this is a surface view of the airway epithelium and because significant epithelial sloughing was not present, we examined epithelial cells almost exclusively. Therefore, if present, labeling of interstitial cells would not be visible through the epithelial layer. As for labeled inflammatory cells observed in the epithelial layer, we believe there are very few to none based on observations of rare events in sections from previous experiments that have used similar BrdU labeling in rats exposed to ozone (32). We can speculate, based on the previous observations of Pino et al. (21) in sections at this same time point after the same dose of ozone exposure, what cell type is being labeled. Pino et al. observed that the ciliated cell is the primary cell undergoing necrosis and that the nonciliated cells (mucous cells in proximal airways and Clara cells in the distal airways) are the proliferative cells.

Our observation of significantly less injury at the CA of vehicle-treated rats compared with capsaicin-treated rats indicates that C-fiber-mediated rapid shallow breathing is protective to the more proximal airways. This observation is consistent with that of Joad et al. (11), who examined the distribution of injury down a single airway path in isolated perfused lungs that were ventilated at different breathing patterns. These observations are also consistent with the observations of Postlethwait et al. (22) in rats and Gerrity et al. (7) in humans that the fractional uptake of ozone of the lower respiratory tract is reduced as f increases. Postlethwait et al. (22) postulate that this f dependence of ozone uptake is the result of f-induced alterations in contact time that directly affect the first-order reactive absorption rate for ozone within the intrapulmonary airways.

The comparison of the distribution of ozone-induced injury in the vehicle-treated rats with the predicted pattern of uptake of ozone (19, 20) provides insight into the validity of these models and allows an examination of the possible mechanisms that may contribute to cellular sensitivity to ozone-induced injury at different regions of the tracheobronchial tree. The ozone uptake model presented by Overton et al. (20) predicts the net and tissue uptake of ozone using two anatomic models of the rat lung. If it is assumed that the severity of injury at any site along an airway path is proportional to the tissue uptake of ozone, then Overton et al.’s model would predict that the terminal bronchioles would show the greatest injury and the most proximal airways the least. Our observed distribution of increasing injury moving down the long airway path fits this pattern. In contrast, our data do not fit the constant net ozone uptake across airway generations that is predicted by Overton et al.’s model, which includes uptake within an airway lining layer as well as within the tissue. If the model of Overton et al. is correct and we assume a similar cellular sensitivity to ozone along an airway path then this would suggest that the generation of reactive products within the airway lining layer is minimal and/or is counterbalanced by antioxidants present in the fluid-lining layer. In this regard, it is interesting that the pattern of BrdU labeling along the long airway path in the present study is opposite of the pattern observed by Joad et al. (11) in isolated perfused lungs ventilated with ozone and using a marker of cellular permeability. These disparate observations may be the result of numerous differences in the preparations used in the two experiments that include 1) conscious spontaneously breathing rats vs. isolated perfused lungs, 2) exposure time (8 vs. 1.5 h), and 3) ventilation pattern (spontaneous ventilation with a minimum frequency of ~95 breaths/min vs. mechanical ventilation at a maximum of 80 breaths/min), and markers of injury (cumulative BrdU label vs. ethidium permeability). Interestingly, in preliminary studies in conscious spontaneously breathing rats (unpublished data), we initially used ethidium permeability as a marker of ozone-induced injury and found very few if any labeled cells at any airway site. Because of our preliminary results using ethidium permeability and our desire to use a measure of injury and repair over the entire exposure period, we used a cumulative label of BrdU. This comparison suggests that one or more factors are associated with the isolated perfused lung preparation that increases the sensitivity of the CA to ozone-induced injury. One potential factor in increasing the sensitivity of the CAs to ozone injury in Joad et al.’s (11) study could be the underperfusion of the conducting airways that occurs in the isolated perfused lung preparation. Indeed, these disparate observations may serve to illustrate the important role of airway blood flow in determining the resistance to injury of the conducting airways to inhaled oxidants.

The effect of abolishing reflex rapid shallow breathing on the distribution of site-specific airway epithelial injury in terminal bronchioles supplied by different
path lengths does not match the pattern that would be expected based on the theoretical models of ozone tissue uptake (19, 20). Overton et al.’s ozone tissue uptake model (20) would predict that, when rapid shallow breathing was attenuated, all terminal bronchioles would show greater injury. Our observations suggest that rapid shallow breathing results in a shift in regional ventilation pattern away from one that favors ventilation of long path airways to one that favors a more even distribution between short and long airway paths. The factors that determine regional flow within the intrapulmonary airway as rapid shallow breathing develops include changes in regional velocity profiles, airway bifurcation geometry, and possibly in regional alveolar pressures related to alteration in thoracic displacement. Schreck (27) examined the flow in latex casts of human lungs and found that the relative amount of flow into two downstream daughter airways is a function of the velocity profile proximal to the bifurcation. In addition, Schreck found that the influence of bifurcation geometry on the relative amount of flow in either daughter branch was greatest when turbulent flow was not present. This relationship was such that the development of turbulence favors a more even distribution of flow between the daughter branches. In the present experiment, peak inspiratory flow after 240 min of ozone inhalation (when \( f \) was maximum in the ozone/vehicle rats) was not significantly different when ozone/vehicle rats were compared with ozone/capsaicin rats (5.08 ± 1.83 vs. 4.99 ± 2.23 ml/s), whereas inspiratory time was significantly shorter in the ozone/vehicle rats (0.16 ± 0.03 vs. 0.26 ± 0.05 s). This flow and timing data demonstrate that, even though the peak inspiratory flow attained by each ozone group was not different, the rate of change in flow was greater in rats that developed rapid shallow breathing. If this more rapid generation of flow results in greater turbulent flow, it would be consistent with our observation that rapid shallow breathing appears to result in a more even distribution of inhaled ozone down airway paths of varying lengths. Although this distribution of regional ventilation may help maintain gas exchange, it appears to also result in greater delivery of ozone to more terminal bronchioles and, as a result, in greater extent of injury to terminal bronchioles throughout the lung.

The observed changes in site-specific injury induced by rapid shallow breathing challenges the notion that rapid shallow breathing is a defensive response acting to protect the deep lung. A critical question to ask is whether similar preferential flow profiles develop in humans who exhibit rapid shallow breathing during ozone inhalation and whether this rapid shallow breathing leads to a greater number of distal airways being injured.

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