Time course of ozone-induced changes in breathing pattern in healthy exercising humans

Edward S. Schelegle,1 William F. Walby,1 and William C. Adams2

1Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, and 2Exercise Biology Program, University of California, Davis, California

Submitted 3 February 2006; accepted in final form 26 October 2006

Schelegle ES, Walby WF, Adams WC. Time course of ozone-induced changes in breathing pattern in healthy exercising humans. J Appl Physiol 102: 688–697, 2007. First published November 2, 2006; doi:10.1152/japplphysiol.00141.2006.—We examined the time course of O3-induced changes in breathing pattern in 97 healthy human subjects (70 men and 27 women). One- to five-minute averages of breathing frequency (fB) and minute ventilation (VE) were used to generate plots of cumulative breaths and cumulative exposure volume vs. time and cumulative exposure volume vs. cumulative breaths. Analysis revealed a three-phase response; delay, no response detected; onset, fB began to increase; response, fB stabilized. Regression analysis was used to identify four parameters: time to onset, number of breaths at onset, cumulative inhaled dose of ozone at onset of O3-induced tachypnea, and the percent change in fB. The effect of altering O3 concentration, VE, atropine treatment, and indomethacin treatment were examined. We found that the lower the O3 concentration, the greater the number of breaths at onset of tachypnea at a fixed ventilation, whereas number of breaths at onset of tachypnea remains unchanged when VE is altered and O3 concentration is fixed. The cumulative inhaled dose of O3 at onset of tachypnea remained constant and showed no relationship with the magnitude of percent change in fB. Atropine did not affect any of the derived parameters, whereas indomethacin did not affect time to onset, number of breaths at onset, or cumulative inhaled dose of O3 at onset of tachypnea but did attenuate percent change in fB. The results are discussed in the context of dose response and intrinsic mechanisms of action.

ozone; oxidants; breathing pattern; tachypnea; kinetics

Despite the extensive literature examining the effects of the acute inhalation of the photochemical air pollutant ozone (O3) in human subjects, little is known about the time course of O3-induced responses. To a large extent, this deficit is due to the design of the majority of human exposure studies that have been used in establishing the air quality standard for O3. In these studies, pulmonary functions are measured before and immediately following the inhalation of 80–400 parts/billion (ppb) O3 for periods of 0.5–6.6 h in combination with light to severe exercise, while breathing pattern and symptom parameters are evaluated during the initial and final 5 min of exposure. Although studies that examine mechanisms have combined multiple interventions to this design to block or modify one or more O3-induced responses, as a result of these studies, several measurable O3-induced responses have been identified, including symptoms of breathing discomfort, reduced inspiratory capacity, rapid shallow breathing during exercise, mild bronchoconstriction, and airway hyperresponsiveness (33).

O3-induced physiological and symptomatic responses can be viewed as being the result of a complex cascade of events. This cascade is composed of multiple interrelated stages that include but are not limited to 1) the inhalation of O3 and those physical factors that determine the distribution of O3 in the respiratory tract, including airway flow patterns and airway geometry (5, 25, 29); 2) the reaction and uptake of O3 within the respiratory tract, as determined by the composition of the airway lining fluid (ALF), including antioxidants and lipid components (3, 7, 8, 11, 23, 24, 32); 3) the interaction of ozonation products with airway epithelial cells leading to oxidant stress and inflammation, including the release of mediators (15, 16, 34); and 4) the activation of airway sensory nerves by inflammatory mediators initiating pulmonary function decrements, rapid shallow breathing, and symptoms (4, 13, 22, 28, 29, 30). Each stage in this cascade has its own kinetics and gain and potentially contributes to the time course and magnitude of O3-induced physiological and symptom responses. Studies examining the time course of ozone-induced responses would improve the ability to partition different stages of this cascade and determine their relative contribution in determining the magnitude of response.

Anecdotally, participants in O3 inhalation studies report a period of exposure that precedes the onset of symptoms, suggesting a delay in onset of O3-induced response. Further supporting the notion that there is a delay in onset of responses are those studies where O3 exposure has been broken into defined intervals. Schelegle et al. (27) report that O3-sensitive subjects who inhaled 200 ppb O3 in two consecutive 40-min intervals did not have significant alterations in pulmonary function and symptoms after the first 40-min segment but did have significant decrements in function and symptoms after the second segment. Folinsbee et al. (10), McDonnell et al. (19), and Adams (2) have reported that, in 6.6-h exposure protocols broken into six 1-h intervals, the inhalation of 80–120 ppb requires 1–3 h for decrements in pulmonary function to develop. These findings support the existence of a delay in onset of O3-induced physiological responses; however, because of the length of the segments used, it is difficult to precisely define the time required to induce a response at any given inhaled dose rate. The ability to define the time of onset or the cumulative inhaled dose of O3 at onset would greatly improve the ability to predict the minimum no effect dose for O3, as well as potentially providing new insights into underlying mechanisms of O3-induced responses.

Address for reprint requests and other correspondence: E. S. Schelegle, Dept. of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, Univ. of California, One Shields Ave., Davis, CA 95616 (e-mail: esschelegle@ucdavis.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
In this analysis, we examined the time course of O₃-induced increases in breathing frequency (fₜ). The data used were collected as part of O₃-inhalation studies in our laboratory over the last 20 yr (21, 26, 27, 29). All studies were done using continuous exercise that produced constant minute ventilations (Vₑ) during the exposure protocols, while the subjects inhaled filtered air (FA) containing O₃ by way of a mouthpiece or facemask that allowed the continuous measurement of fₜ and tidal volume (V₉). The O₃ concentrations used in these studies ranged from 120 to 350 ppb and are in the range of concentrations of other studies that have been used to set national air-quality criteria for O₃. This range closely approximates maximum 1-h mean O₃ concentrations in the most polluted urban areas in the United States (33). As a reference, the current United States Environmental Protection Agency air-quality standard for O₃ is an 8-h average of 80 ppb or 120 ppb for a 1-h average. The United States National Institute for Occupational Safety and Health short-term exposure limit is 300 ppb. We determined the time to onset, number of breaths at onset, and cumulative inhaled dose of O₃ at onset of O₃-induced increase in fₜ in 87 normal exercising male and female subjects. Because several of these subjects completed multiple O₃-exposure protocols, a total of 248 raw data records were evaluated and used in this analysis. The relationship between the derived time course parameters, individual subject characteristics, and O₃-induced decrements in forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV₁) were examined using stepwise linear regression and correlation analyses. Also available within this data were data subsets that permitted the examination of the effects of altering O₃ concentration (350 vs. 200 ppb and 300 vs. 180 ppb), Vₑ (70 vs. 50 l/min), atropine treatment, and indomethacin treatment.

METHODS

The data used in this analysis were derived from the raw data records of six studies that were conducted in the University of California Davis Human Performance Laboratory from 1986 to 2005 and were approved by the University of California Davis, Institutional Review Board. A brief description of each study is given in Table 1. Results from four of these studies have been previously reported in the peer-reviewed literature (21, 26, 27, 29). In total, files were available for 187 healthy human subjects (146 men and 41 women), 18–45 yr of age. Within these files, at least one complete data record for an O₃-exposure protocol was available for 87 subjects (67 men and 20 women). As a result of the design of the studies in which they participated, 46 subjects participated in multiple FA and O₃-inhalation protocols. As a result, a total of 248 records were evaluated and used in this analysis. Data from each study were considered a data subset. These data subsets were used to examine the effects of O₃ concentration (350 vs. 200 ppb and 300 vs. 180 ppb), Vₑ (70 vs. 50 l/min), atropine treatment, and indomethacin treatment on the derived end points.

Exposure and measurements. The same ozone generation and delivery, ventilation monitoring, and pulmonary function equipment was used in all the studies included in this analysis. All equipment was continuously housed, and experiments were performed in the University of California Davis, Human Performance Laboratory, Davis, CA. During each protocol, subjects breathed through a silicone mouthpiece (58 subjects, studies 1–4; see Table 1) or wore a Teflon-coated silicone facemask (29 subjects, studies 5 and 6; see Table 1) attached to a two-way respiratory valve (Hans Rudolph, Kansas City, MO) through which they inhaled FA or O₃ in FA while exercising on a cycle ergometer (model 845, Quinton Instrument, Bothell, WA, or model 830, Monarch Exercise, Vansbro, Sweden). To examine whether there was an effect of mouthpiece vs. facemask exposure, we analyzed a subset of the data in which we controlled for total inhaled dose and inhaled dose rate. This analysis showed that there were no significant differences between mouthpiece (n = 38) and facemask (n = 28) for any of the measured end points in this study (comparison not shown).

Air was filtered by a Barneby-Cheney charcoal filter (Columbus, OH) before being inhaled by the subject. Details on the O₃-inhalation system are described elsewhere (9). In brief, FA was blended with O₃ generated by passing pure oxygen through an ozonizer (Type II, Sander). O₃ was continuously sampled from the inspiratory side of the

Table 1. Summary of studies that acted as the source of data for the time-course analysis of O₃-induced rapid shallow breathing in healthy adult human subjects

<table>
<thead>
<tr>
<th>Study No.</th>
<th>Subjects</th>
<th>Brief Description</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14 men</td>
<td>Mechanisms of ozone-induced responses in healthy human subjects. 10 protocols (FA and 350 ppb O₃ at 60 l/min for 1 h with atropine, atropine sham, no drug, indomethacin, and indomethacin placebo).</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>67 men</td>
<td>Characterization of O₃ sensitivity. 1 protocol (350 ppb O₃ at 60 l/min); duration is 1 h.</td>
<td>Unpublished</td>
</tr>
<tr>
<td>3</td>
<td>40 men; 20 O₃ sensitive and 20 O₃ nonsensitive</td>
<td>Plasma prostaglandins and ozone sensitivity. 3 protocols (FA, and 200 and 350 ppb O₃); two 40-min intervals at 50 l/min.</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>20 men; 20 women</td>
<td>Male and female responses to ozone inhalation consequent to the same absolute and relative minute ventilation. Men: 6 protocols (0, 180, and 300 ppb O₃ at 50 and 70 l/min) for 1 h. Women: 9 protocols (0, 180, and 300 ppb O₃ at 50 l/min for 1 h during the midluteal, late luteal, and early follicular phases of their menstrual cycles.</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>11 men; 11 women</td>
<td>Effect of airway anesthesia on ozone-induced breathing pattern, pulmonary function, and subjective symptom responses. Four 80-min protocols: 1) FA with saline. 2) FA with tetracaine. 3) 300 ppb O₃ with saline. 4) 300 ppb O₃ with tetracaine. In each exposure with face masks, subjects exercised at a minute ventilation of ~30 l/min⁻¹·m⁻² BSAs for 50 min, followed by tetracaine (or saline) rebreathing through a nebulizer for 5 min during a 15-min rest, followed by a 15-min exercise bout at ~30 l/min⁻¹·m⁻².</td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>4 men; 4 women</td>
<td>Breath condensate as a noninvasive measurement of ozone-induced airway inflammation in humans. Two 1-h exposures; minute ventilation was 50 l/min. O₃ concentrations for the exposures were 0 and 350 ppb.</td>
<td>Unpublished</td>
</tr>
</tbody>
</table>

FA, filtered air; ppb, parts/billion; BSA, body surface area.
Ozone-induced changes in breathing pattern

J Appl Physiol • VOL 102 • FEBRUARY 2007 • www.jap.org

Hans-Rudolph valve and analyzed using a Dasibi O3 monitor (model 1003-AH, Dasibi Environmental, Glendale, CA). The Dasibi monitor was calibrated before and after each study using the ultraviolet absorption photometric method at the California National Primate Research Center, University of California, Davis. In those studies where subjects completed multiple protocols, consecutive O3-exposure protocols were separated by at least 96 h.

Expired air passed through a 5-liter stainless steel mixing and sampling chamber and into a turbotachometer (VMM-2, Interface Associates, Aliso Viejo, CA) that was used to measure expired air flow. To obtain 15-s averages of $V_e$, $V_t$, and $f_B$, the output from the turbotachometer and a temperature thermistor located in the mixing chamber were interfaced with a digital acquisition system.

Atropine and indomethacin treatment. Fourteen male subjects inhaled atropine sulfate aerosol (0.039 mg/kg, with a range of 0.034–0.046 mg/kg) 20 min before performing preexposure pulmonary function tests. Atropine sulfate aerosols were generated using a modified ultrasonic nebulizer (Mist-O-Gen). The nebulizer was modified so that the expired aerosol was trapped in an attached filter. The amount of atropine delivered was determined by weighing the nebulizer chamber plus filter assembly before and after aerosol inhalation. The dose and time of atropine administration was such that it would result in peak bronchodilation at the time of the preexposure pulmonary function tests and then plateau for the remainder of the time needed to complete a 1-h protocol and postexposure pulmonary function tests. All subjects reported the symptom of dry mouth with atropine inhalation, with 11 subjects also reporting transient dizziness varying in degree from mild to severe. Two subjects also reported mild headache during the atropine inhalation protocols.

Subjects ingested indomethacin (Indocin SR 75, 75 mg of indomethacin, Merck, Sharp, and Dohme, West Point, PA) twice daily with morning and evening meals for 6 days. Subjects inhaled O3 either on day 3 or 6 of this treatment regimen (26).

Derived data. $f_B$, $V_t$, and $V_e$ data were available as 1- or 5-min averages and were transcribed from computer printouts into the database along with pre- and postexposure values for FVC and FEV1 for five of seven studies used. Data stored on computer disks were available for the other two studies. To analyze the time course of $f_B$, $V_t$, and $V_e$ responses during a single exposure protocol, the number of breaths and the volume exhaled during each averaging period was calculated. From these data, the cumulative number of breaths and exhaled volume were calculated for each time point. The cumulative number of breaths and exhaled volume were then plotted against time, and cumulative exhaled volume was plotted against cumulative number of breaths. An example of one of these plots is shown in Fig. 1 for a subject who completed a FA and two O3-inhalation protocols (200 and 350 ppb). It should be noted that the slope of the cumulative number of breaths vs. time is equal to $f_B$ (Fig. IA), the slope of the cumulative exhaled volume vs. time is equal to $V_e$ (Fig. IB), and the slope of cumulative exhaled volume vs. cumulative number of breaths is equal to $V_t$ (Fig. 1C). After several of these plots were examined, it became apparent that it was possible to detect the time of onset of O3-induced tachypnea and decreasing $V_t$. It also became apparent that the plot of cumulative breaths vs. time was best approximated by two intersecting straight lines. Time to onset of tachypnea was derived for each O3 inhalation protocol by using regression analysis in combination with visual examination of each plot. In brief, time to onset of tachypnea was determined by using an iterative process involving least squares linear regression (Excel X, Microsoft) of cumulative breaths and time data. In the first iteration, the first region (region 1) of the data that was fitted included the 5-min time point plus the next two time points. The second region (region 2) that was fitted began at the time point 5 min greater than the last time in region 1 and included all the time points greater than this to the end of the protocol. In the next iteration, a time point was added to region 1 and subtracted from region 2. This iterative process was continued until region 2 consisted of the data from the last 5 min of the protocol. With each iteration step, the slope, intercept, and correlation coefficient were calculated for each region. In addition, the difference in slope of regions 1 and 2 and the
average correlation coefficient of regions 1 and 2 were calculated. The highest point value of region 1 where the maximum in the correlation coefficient of region 1, the average correlation coefficient of regions 1 and 2, and the difference in slopes of region 1 and 2 occurred was determined and averaged to obtain the estimated time to onset of tachypnea. This value was then confirmed visually by examining each plot of cumulative breaths vs. time. Those data in which the above criteria were not met by the last iteration and on visual inspection appeared to be a straight line were considered not to have an inflection point and not to contain an onset of tachypnea. These data were then treated similar to the data obtained during FA inhalation (see below).

Once time to onset of tachypnea was determined, an additional six parameters were derived from the data: number of breaths to onset of tachypnea, the cumulative inhaled dose of O3 in micrograms at the onset of tachypnea, the slope before the onset of tachypnea (S1), the slope after the onset of tachypnea (S2), the average Vt before the onset of tachypnea (Vt1), and the average Vt after the onset of tachypnea (Vt2). Five parameters were derived for each FA inhalation protocol: total number of breaths (Nbt), the slope before the last 10 min of the protocol (S1), the slope in the last 10 min of the protocol (S2), the average VT before the last 10 min of the protocol (Vt1), and the average VT in the last 10 min of the protocol (Vt2). Five parameters were derived for each FA inhalation protocol: total number of breaths (Nbt), the slope before the last 10 min of the protocol (S1), the slope in the last 10 min of the protocol (S2), the average VT before the last 10 min of the protocol (Vt1), and the average VT in the last 10 min of the protocol (Vt2).

Statistical analysis. Stepwise regression (Statview, SAS) was used to examine the relationship between derived parameters (time to onset of tachypnea and percent change in fB, VT, FVC, FEV1, and FEV1/FVC), subject characterization (age, height, weight, body surface area, baseline FVC, and baseline FEV1/FVC), and exposure (O3 concentration, O3 inhaled dose rate, total inhaled O3 dose, fB before onset of tachypnea, Vt before onset of tachypnea, and length of exposure) data. Inclusion criteria for independent variables in the regression models was $P < 0.05$ for each variable’s individual coefficient.

Correlation analysis (Statview, SAS) was used to examine the relationship between the protocol mean values for cumulative inhaled dose of O3 at onset of tachypnea, inhaled dose rate, percent change in fB, and the other derived parameters (percent change in FVC, FEV1, FEV1/FVC, Vt, and time to the onset and number of breaths at onset of tachypnea). Data from the atropine and indomethacin treatment protocols were not included in the stepwise regression or correlation analysis.

Following these analyses, the effect of O3 concentration, Vt, atropine treatment, and indomethacin treatment was examined in subsets of the database using multivariate ANOVA or ANOVA with repeated measures (Statview, SAS). Specific mean differences were examined using repeated paired t-tests with Bonferroni correction (Statview, SAS). Level of significance was set at $P < 0.05$ for all comparisons.

RESULTS

Subject characterization data. All subjects were healthy, nonsmoking, physically active adults without a history of asthma. Subjects that reported having allergic rhinitis were asymptomatic and not on medication at the time of study. Histograms for age (yr), height (cm), body weight (kg), body surface area (m²), baseline FVC (liters), baseline FEV1/FVC (%) for male and female subjects are shown in Fig. 2. There was no significant difference in age and FEV1/FVC% between male and female subjects. Male subjects were larger on average than female subjects, as indicated by significantly

Fig. 2. Histograms showing the distribution of age (yr), height (cm), body weight (kg), body surface area (BSA; m²), baseline forced vital capacity (FVC; liters), and baseline forced expiratory volume in 1 s (FEV1/FVC (%)) for male (M) and female (F) subjects. Values are means ± SD.
greater height, body weight, body surface area, and baseline FVC in the male subjects compared with the female subjects. Interestingly, there was no significant difference in O₃-induced decrements in FVC, FEV₁, FEV₁/FVC, percent change in VT, percent change in fB, and cumulative inhaled dose of O₃ at onset of tachypnea in comparably exposed male and female subjects. As a result, no further comparison between male and female data were made.

In 20 of the 157 O₃ exposure protocols examined an inflection point in fB indicating the onset of tachypnea could not be detected. Most often (19 of 20 cases) this occurred in the protocols utilizing a lower V̇E (50 l/min) and O₃ concentration (200 or 180 ppb). Because it was not possible to accurately derive time to, number of breaths at, and cumulative inhaled dose of O₃ at onset of tachypnea from these protocols, they were excluded from the statistical analysis. It is interesting to note that in every one of these cases in which an inflection point was not detected the cumulative inhaled dose for the entire protocol was lower than the cumulative inhaled dose of O₃ at onset of tachypnea calculated in the same subjects in protocols where an inflection point in fB was detected (Table 2).

**Stepwise regression.** Histograms of baseline fB and VT, as well as V̇E and V̇E/BSA from protocols that were used in the stepwise regression analysis are shown in Fig. 3. Stepwise regression resulted in a significant (P < 0.0001; r = 0.663) association between the time to onset of tachypnea (TOT) and body weight (BWT), O₃ concentration ([O₃]), fB before onset of tachypnea (fBBOT) and VT before onset of tachypnea (VTBOT) (Eq. 1; Fig. 4A). Body surface area could be substituted in this relationship without affecting the P value. In addition, there was a significant (P < 0.0001; r = 0.406 and 0.427) association between both the percent change in fB (%fB) and VT (%VT) with age, baseline FVC (FVCbaseline), and O₃ inhaled dose rate (DR) (Eqs. 2 and 3; Fig. 4, B and C).

\[
\text{T}_{\text{OT}} = 115.9 + 0.4 \text{BWT} - 114.8 \left( 0.4 \text{fB}/\text{fBBOT} + 21.7 \text{VT}/\text{VTBOT} \right) \quad (1)
\]

\[
\% \Delta \text{f}_B = -2.0 - 0.8 \text{age} + 5.9 \text{FVC}_\text{baseline} + 0.7 \text{DR} \quad (2)
\]

\[
\% \Delta \text{VT} = -3.7 + 0.4 \text{age} - 2.9 \text{FVC}_\text{baseline} - 0.4 \text{DR} \quad (3)
\]

Interestingly, the percent change in FVC (%FVC) and FEV₁ (%FEV₁) also resulted in a best fit with equations that included O₃ inhaled dose rate (P = 0.0002 and 0.0010, respectively) (Eqs. 4 and 5), whereas percent change in FEV₁/FVC (%FEV₁/FVC) was best fit with an equation that included O₃ concentration (P = 0.0002) (Eq. 6).

### Table 2. *Values for the percent change in FVC, FEV₁, FEV₁/FVC, VT, and fB*  

<table>
<thead>
<tr>
<th>Exposure</th>
<th>%ΔFVC</th>
<th>%ΔFEV₁</th>
<th>%ΔFEV₁/FVC</th>
<th>%ΔVT</th>
<th>%ΔfB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtered Air</td>
<td>2.91±5.12</td>
<td>4.13±2.87</td>
<td>1.34±4.46</td>
<td>-2.83±4.80</td>
<td>3.16±5.21</td>
</tr>
<tr>
<td>Ozone</td>
<td>-1.70±4.52</td>
<td>-2.96±6.93</td>
<td>-1.32±4.24</td>
<td>-3.48±3.39</td>
<td>3.73±3.66</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 20) of protocols where subjects did not attain a cumulative inhaled dose of O₃ sufficient to induce the onset of tachypnea. %Δ, Percent change; FVC, forced vital capacity; VT, tidal volume; FEV₁, forced expiratory volume in 1 s; fB, breathing frequency.
\[ \% \Delta \text{FVC} = -48.3 + 0.3 \text{height} - 0.6 \text{DR} \]  \hspace{1cm} (4)
\[ \% \Delta \text{FEV}_1 = -80.9 + 0.8 \text{age} \]
\[ - 5.1 \text{FVC}_{\text{baseline}} + 0.5 \text{height} - 0.6 \text{DR} \]  \hspace{1cm} (5)
\[ \% \Delta \text{FEV}_1/\text{FVC} = -23.1 + 0.7 \text{age} \]
\[ - 3.0 \text{FVC}_{\text{baseline}} + 0.4 \text{FEV}_1/\text{FVC}_{\text{baseline}} - 34.5 [\text{O}_3] \]  \hspace{1cm} (6)

**Correlation analysis.** Percent change in \( f_B \) was significantly correlated with percent change in FVC (Fig. 4A), FEV\(_1\), and \( V_T \), but not percent change in \( \text{FEV}_1/\text{FVC} \) (Table 3). The cumulative inhaled dose of \( \text{O}_3 \) at onset of tachypnea, in contrast, was not correlated with percent change in FVC, \( \text{FEV}_1\), \( V_T \), and \( f_B \), but was correlated with percent change in \( \text{FEV}_1/\text{FVC} \), time to onset of tachypnea, and number of breaths at onset of tachypnea (Table 3). This indicates that there exists some association between the magnitude of the tachypnic response and the pulmonary function responses that are related to \( \text{O}_3 \)-induced reductions in inspiratory capacity but not \( \text{O}_3 \)-induced bronchoconstriction. In contrast, there was little or no association between cumulative inhaled dose of \( \text{O}_3 \) at onset of tachypnea and the magnitude of pulmonary function responses (percent change in FVC and \( \text{FEV}_1 \)), time to onset of tachypnea, number of breaths at onset of tachypnea, inhaled dose rate (Fig. 5A), percent change in \( V_T \), and percent change in \( f_B \) (Fig. 5B), whereas there was a significant association between cumulative inhaled dose of \( \text{O}_3 \) at onset of tachypnea and percent change in \( \text{FEV}_1/\text{FVC} \) (Table 3). In comparison, inhaled dose rate was correlated with percent change in FVC, \( \text{FEV}_1\), \( f_B \) (Fig. 5C), \( V_T \), time to onset of tachypnea, and number of breaths at onset of tachypnea but not cumulative inhaled dose of \( \text{O}_3 \) at onset of tachypnea (Fig. 5C) or percent change in \( \text{FEV}_1/\text{FVC} \) (Table 3).

In addition, percent change in \( f_B \) was correlated with percent change in FVC (Fig. 5D) and \( V_T \) (Table 3).

**Ozone concentration.** Two subsets of the data were used to examine the effect of changing \( \text{O}_3 \) concentration on time to onset of tachypnea, number of breaths at onset of tachypnea, cumulative inhaled dose of \( \text{O}_3 \) at onset of tachypnea, and percent change in \( f_B \). In the first (study 3 in Table 1), subjects inhaled either 350 or 200 ppb \( \text{O}_3 \) at a \( V_E \) of 50 l/min in two 40-min bouts of exercise separated by a 10-min rest period, during which forced expiratory maneuvers were preformed and a blood sample was collected. Mean values for pulmonary function and \( f_B \) parameters are given in Table 4. In the second subset, where the effect of \( \text{O}_3 \) concentration was examined (study 4 in Table 1), subjects inhaled either 180 or 300 ppb \( \text{O}_3 \) at a \( V_E \) of 70 l/min for 60 min. In both subsets, inhaling \( \text{O}_3 \) (180, 200, 300, or 350 ppb) resulted in significant decrements in FVC and \( \text{FEV}_1 \) along with significant increases in percent change in \( f_B \) (\( P < 0.05 \)). In addition, increasing the inhaled \( \text{O}_3 \) concentration from 180 to 300 ppb or 200 to 350 ppb resulted in significantly greater decrements in FVC and \( \text{FEV}_1 \) and significantly greater increases in \( f_B \). The effect of increasing inhaled \( \text{O}_3 \) concentration on \( \text{FEV}_1/\text{FVC} \) was more variable (Table 4). Increasing the inhaled \( \text{O}_3 \) concentration resulted in significant decreases in time to onset of tachypnea and number of breaths at onset of tachypnea. In contrast, increasing the inhaled \( \text{O}_3 \) concentration did not result in a significant change in cumulative inhaled dose of \( \text{O}_3 \) at onset of tachypnea (Table 4). Importantly, increasing \( \text{O}_3 \) concentration not only decreased the mean values of time to onset of tachypnea and number of breaths at onset of tachypnea, but also decreased the minimums for these parameters. For example, minimum time to onset of tachypnea decreased from 40.0 to 19.5 min and minimum number of breaths at onset of tachypnea decreased from 1,179 to 511 breaths for the 200 vs. 350 ppb \( \text{O}_3 \) comparison.

\( V_E \). A subset of the data (study 4 in Table 1) was used to examine the effect of changing \( V_E \) on time to onset of tachypnea, number of breaths at onset of tachypnea, cumulative inhaled dose of \( \text{O}_3 \) at onset of tachypnea and percent change in \( f_B \). In this subset, subjects inhaled 300 ppb \( \text{O}_3 \) at a \( V_E \) of either
50 or 70 l/min for 60 min. Inhaling 300 ppb O₃ at a Vₑ of either 50 or 70 l/min resulted in significant decrements in FVC, FEV₁, and FEV₁/FVC along with significant increases in fₑ (Table 4). Increasing Vₑ resulted in significantly greater decrements in FVC and significantly greater increases in fₑ. In addition, increasing Vₑ resulted in a significant decrease in time to onset of tachypnea, but not in number of breaths at onset of tachypnea or cumulative inhaled dose of O₃ at onset of tachypnea (Table 4).

**Atropine and indomethacin treatment.** A subset of the data was used to examine the effect of the muscarinic receptor antagonist, atropine, or the cyclooxygenase inhibitor, indomethacin, treatment (study 1 in Table 1) on time to onset of tachypnea, number of breaths at onset of tachypnea, cumulative inhaled dose of O₃ at onset of tachypnea, and percent change in fₑ. In this subset, subjects inhaled 350 ppb O₃ at a Vₑ of 50 l/min for 60 min after receiving no treatment, atropine aerosol, or oral indomethacin. Inhaling 350 ppb O₃ with no treatment resulted in significant decrements in FVC, FEV₁, and FEV₁/FVC, along with significant increases in fₑ (Table 5). Atropine treatment significantly reduced the O₃-induced decrements in FEV₁/FVC but did not significantly affect O₃-induced decrements in FVC and FEV₁ or increases in fₑ. In contrast, indomethacin treatment significantly reduced the O₃-induced decrements in FVC and FEV₁ and increases in fₑ, but did not significantly affect O₃-induced decrements in FEV₁/FVC. Neither atropine nor indomethacin treatment significantly affected time to onset of tachypnea, number of breaths at onset of tachypnea, or cumulative inhaled dose of O₃ at onset of tachypnea. The reported effects of atropine treatment indicate that time to onset of tachypnea, number of breaths at onset of tachypnea, and percent change in VT and fₑ are independent of O₃-induced bronchoconstriction. The reported effects of indomethacin treatment indicate that time to onset of tachypnea, number of breaths at onset of tachypnea, and cumulative inhaled dose of O₃ at onset of tachypnea are independent of the release or production of cyclooxygenase metabolites, whereas percent change in VT and fₑ are at least in part dependent on the production of cyclooxygenase metabolites.

**DISCUSSION**

This is the first systematic examination of the time course of onset of an O₃-induced functional response in human subjects, and it indicates that the inhalation of high ambient concentrations of O₃ does not induce an immediate alteration of airway function after one to two breaths but requires many more. The approach used allowed us to determine the time to onset of tachypnea, number of breaths at onset of tachypnea, and cumulative inhaled dose of O₃ at onset of tachypnea for each protocol and relate these parameters to the magnitude of response under multiple condi-

---

**Table 3. Results of correlation analysis**

<table>
<thead>
<tr>
<th></th>
<th>%ΔFVC</th>
<th>%ΔFEV₁</th>
<th>%ΔFEV₁/FVC</th>
<th>%ΔVT</th>
<th>Tos</th>
<th>Nbos</th>
<th>%Δfₑ</th>
<th>Dos</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR</td>
<td>0.709</td>
<td>0.713</td>
<td>0.217</td>
<td>0.745</td>
<td>0.829</td>
<td>0.890</td>
<td>0.739</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>(0.030)</td>
<td>(0.029)</td>
<td>(0.590)</td>
<td>(0.019)</td>
<td>(0.004)</td>
<td>(0.001)</td>
<td>(0.020)</td>
<td>(0.775)</td>
</tr>
<tr>
<td>Dos</td>
<td>0.018</td>
<td>0.397</td>
<td>0.714</td>
<td>0.142</td>
<td>0.390</td>
<td>0.247</td>
<td>0.231</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.964)</td>
<td>(0.304)</td>
<td>(0.028)</td>
<td>(0.726)</td>
<td>(0.313)</td>
<td>(0.537)</td>
<td>(0.564)</td>
<td></td>
</tr>
<tr>
<td>%Δfₑ</td>
<td>0.733</td>
<td>0.646</td>
<td>0.037</td>
<td>0.987</td>
<td>0.419</td>
<td>0.574</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.022)</td>
<td>(0.060)</td>
<td>(0.927)</td>
<td>(-0.001)</td>
<td>(0.274)</td>
<td>(0.109)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are the correlation coefficient, with its associated P value in parentheses. Results of correlation analysis between cumulative inhaled dose of O₃ at onset of tachypnea (Dos), inhaled dose rate (DR), time to onset of tachypnea (Tos), number of breaths at onset of tachypnea (Nbos), and percent changes in breathing frequency (%Δfₑ), functional residual capacity (%ΔFVC), forced expiratory volume in 1 s (%ΔFEV₁), FEV₁/FVC (%ΔFEV₁/FVC), and tidal volume (%ΔVT).

---

**Fig. 5. Scatterplots of subset data mean values of cumulative inhaled dose of O₃ at onset of tachypnea (Dos) vs. inhaled dose rate (DR) (A), Dos vs. percent change in fₑ (%Δfₑ) (B), %Δfₑ vs. DR (C), and percent change in FVC (%ΔFVC) vs. %Δfₑ (D).** Lines represent best-fit lines.
tachypnea remained constant across all exposure conditions and \( V\dot{E} \) is altered and \( O_3 \) concentration is fixed. Even more intriguing is the observation that cumulative inhaled dose of \( O_3 \) at onset of tachypnea is exceeded the tachypnic ventilatory response becomes fixed is suggestive that the magnitude of respiratory capacity, since residual volume is not significantly elevated by \( O_3 \) inhalation (1, 31, 33). \( O_3 \)-induced decrements in \( FEV_1 \) are the result of the decreased inspiratory capacity and to a lesser extent mild bronchoconstriction (4, 33) since treatment with atropine in this and another study (4) abolishes \( O_3 \)-induced increases in airway resistance while only producing a mild improvement in \( FEV_1 \). In turn, reflex inhibition of the ability to inspire has been implicated in both \( O_3 \)-induced decreases in inspiratory capacity (13, 22) and rapid shallow breathing (22, 29). Hazucha et al. (13) found that \( O_3 \)-induced decreases in inspiratory capacity and rapid shallow breathing are not the result of a decrease in dynamic or static lung compliance or respiratory muscle strength (13). Passannante et al. (22) using the opioid-receptor antagonist, sufentanil, were able to reverse \( O_3 \)-induced decrements in \( FEV_1 \), implicating the involvement of airway C-fibers that are known to be involved in \( O_3 \)-induced rapid shallow breathing in rats (30) and dogs (28). More recently, Schlegle et al. (29), observed that the inhalation of an aerosol of the local anesthetic, tetracaine, significantly reduced and in some cases completely abolished \( O_3 \)-induced symptoms of breathing discomfort, whereas this treatment did not significantly affect \( O_3 \)-induced decreases in inspiratory capacity and rapid shallow breathing. This observation confirms that \( O_3 \)-induced decreases in inspiratory capacity and rapid shallow breathing are independent of symptoms of breathing discomfort and are not the result of a subject’s unwillingness to take complete or deep breaths. Taken together, the findings of these mechanistic studies indicate a common neural origin for \( O_3 \)-induced decreases in inspiratory capacity and rapid shallow breathing that would imply that they would follow a similar time course. This possibility is supported by our observation that, when cumulative inhaled dose-response relationships in human subjects have related changes in FVC and inspiratory capacity, but do not significantly affect \( O_3 \)-induced decreases in \( FEV_1 \) or \( VT \), and \( f_B \) of subject subsets used to examine the effect of \( O_3 \) concentration and ventilation on the time course of \( O_3 \)-induced rapid shallow breathing in healthy human subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>[( O_3 )], ppb</th>
<th>( V_l ), l/min</th>
<th>( %\Delta FVC )</th>
<th>( %\Delta FEV/FVC )</th>
<th>( %\Delta VT )</th>
<th>( %\Delta f_B )</th>
<th>( T_os ), min</th>
<th>Nbos</th>
<th>Dos, ( \mu g )</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>50.25 ± 2.85</td>
<td>2.95 ± 3.46</td>
<td>2.81 ± 3.58</td>
<td>1.80 ± 0.18</td>
<td>-7.40 ± 5.78</td>
<td>28.18 ± 3.47</td>
<td>8.37 ± 6.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>49.53 ± 2.72</td>
<td>-8.92 ± 11.91*</td>
<td>-3.63 ± 5.21</td>
<td>1.77 ± 0.20</td>
<td>-20.31 ± 10.70*</td>
<td>28.37 ± 3.24</td>
<td>28.00 ± 20.22*</td>
<td>57.4 ± 11.3</td>
<td>1,630.00 ± 374</td>
</tr>
<tr>
<td>300</td>
<td>49.43 ± 3.89</td>
<td>-17.99 ± 17.86†</td>
<td>-1.79 ± 7.28</td>
<td>1.81 ± 0.23</td>
<td>-26.06 ± 11.54†</td>
<td>27.70 ± 3.65</td>
<td>38.74 ± 24.33†</td>
<td>32.6 ± 5.5</td>
<td>906 ± 235</td>
</tr>
<tr>
<td>10 men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>68.94 ± 2.42</td>
<td>-0.35 ± 3.55</td>
<td>2.13 ± 5.00</td>
<td>2.29 ± 0.22</td>
<td>-4.42 ± 3.55</td>
<td>30.45 ± 3.66</td>
<td>4.75 ± 3.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>68.08 ± 2.84</td>
<td>-6.39 ± 5.66*</td>
<td>-3.75 ± 7.04*</td>
<td>2.13 ± 0.28</td>
<td>-14.65 ± 5.80</td>
<td>32.58 ± 4.76</td>
<td>17.65 ± 8.00*</td>
<td>34.9 ± 11.3</td>
<td>1,252 ± 486</td>
</tr>
<tr>
<td>300</td>
<td>68.42 ± 2.3</td>
<td>-22.93 ± 13.42†</td>
<td>-2.98 ± 5.36†</td>
<td>2.17 ± 0.40</td>
<td>-30.87 ± 7.78</td>
<td>32.59 ± 6.36</td>
<td>46.33 ± 16.71†</td>
<td>22.0 ± 9.0</td>
<td>703 ± 418</td>
</tr>
<tr>
<td>500</td>
<td>49.55 ± 1.93</td>
<td>-15.71 ± 10.97†</td>
<td>-8.35 ± 7.09†</td>
<td>1.72 ± 0.21</td>
<td>-20.37 ± 6.29†</td>
<td>29.15 ± 0.21</td>
<td>26.28 ± 9.84†</td>
<td>37.2 ± 10.5</td>
<td>1,073 ± 312</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( V_l \), minute ventilation; [\( O_3 \)], \( O_3 \) concentration; ppb, parts/billion. *Significant difference from filtered air (0 ppb \( O_3 \)). †Significant difference from 200 or 180 ppb \( O_3 \). §Significant difference between 300 ppb \( O_3 \) at 70 l/min and 300 ppb \( O_3 \) at 50 l/min. P value set at 0.05.

Table 5. Mean values for \( \%\Delta FVC \), \( \%\Delta FEV/FVC \), \( \%\Delta VT \), and \( \%\Delta f_B \) of subject subsets used to examine the effect of atropine and indomethacin treatment on the time course of \( O_3 \)-induced rapid shallow breathing in healthy human subjects.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>[( O_3 )], ppb</th>
<th>( V_l ), l/min</th>
<th>( %\Delta FVC )</th>
<th>( %\Delta FEV/FVC )</th>
<th>( %\Delta VT )</th>
<th>( %\Delta f_B )</th>
<th>( T_os ), min</th>
<th>Nbos</th>
<th>Dos, ( \mu g )</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>50.25 ± 2.85</td>
<td>-1.17 ± 3.03</td>
<td>3.38 ± 4.85</td>
<td>1.77 ± 0.13</td>
<td>-6.98 ± 6.08</td>
<td>33.42 ± 3.54</td>
<td>7.92 ± 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>50.13 ± 5.13</td>
<td>-18.83 ± 15.95*</td>
<td>-10.92 ± 16.77*</td>
<td>1.76 ± 0.22</td>
<td>-26.50 ± 9.70*</td>
<td>32.83 ± 1.11</td>
<td>38.59 ± 21.2*</td>
<td>27.4 ± 7.3</td>
<td>894 ± 280</td>
</tr>
<tr>
<td>Atropine</td>
<td>50.76 ± 2.69</td>
<td>-22.55 ± 17.76*</td>
<td>-0.45 ± 9.92*</td>
<td>1.83 ± 0.17</td>
<td>-27.00 ± 10.66*</td>
<td>31.81 ± 2.89</td>
<td>40.05 ± 23.5*</td>
<td>25.2 ± 8.6</td>
<td>802 ± 299</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>50.88 ± 2.70</td>
<td>-8.49 ± 8.56*</td>
<td>-4.35 ± 8.85*</td>
<td>1.80 ± 0.13</td>
<td>-19.74 ± 6.61*</td>
<td>31.67 ± 2.41</td>
<td>25.37 ± 10.39†</td>
<td>29.1 ± 8.3</td>
<td>907 ± 268</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significant difference from filtered air (0 ppb \( O_3 \)).†Significant difference from 350 ppb \( O_3 \) without treatment. P value set at 0.05.
dose of O₃ at onset of tachypnea was not achieved, FVC and FEV₁ decrements were mild or not present (Table 2). Another intriguing result of our data analysis was that, once established, O₃-induced increases in fB were proportionally related to inhaled O₃ dose rate or the product of fB and O₃ concentration. Again the question to be asked is, do O₃-induced decrements in FVC and FEV₁ show the same relationship with inhaled dose rate? The result of stepwise regression and correlation analysis on our data set would imply that this is the case. Stepwise regression resulted in inhaled dose rate being included in the best-fit models for O₃-induced percent change in fB, fL, FVC, and FEV₁, even when the total inhaled O₃ dose (‘effective dose’) is included as a possible independent variable in the analysis. Although correlation analysis resulted in significant correlations between percent change in FEV₁ and percent change in fB, and inhaled dose rate. It is possible that the apparent increased weight given O₃ concentration in predicting FEV₁ decrements in previous studies (1, 14, 31) is the result of the fact that, as O₃ concentration increases at any fixed VE (i.e., increasing dose rate), the number of subjects who would develop FEV₁ decrements due to the effect of delay to onset and the magnitude of FEV₁ decrements in proportion to dose rate would both increase. As a result, the increasing mean FEV₁ decrement with increasing O₃ concentration would reflect an increasing number of subjects exhibiting a response, as well as an increased response in those subjects that responded or would have responded at a lower concentration of O₃. Consistent with this scenario is McDonnell et al.’s (20) published frequency distribution of percent change in FEV₁ as O₃ concentration increases.

Three observations are consistent with the notion that the underlying intrinsic factors that determine the magnitude of the delay phase and response phase are independent. First, time to onset of tachypnea, number of breaths at onset of tachypnea, and cumulative inhaled dose of O₃ at onset of tachypnea are only poorly correlated, if at all, with percent change in fB, indicating that the length of the delay phase for a given subject does not predict the magnitude of the tachypnic response at any fixed inhaled dose rate. Second, although altering inhaled dose rate by changing O₃ concentration or VE resulted in related changes in percent change in fB, these alterations did not significantly affect cumulative inhaled dose of O₃ at onset of tachypnea. Third, indomethacin treatment, while significantly attenuating percent change in fB, did not change time to onset of tachypnea, number of breaths at onset of tachypnea, or cumulative inhaled dose of O₃ at onset of tachypnea. This posits that our observations are consistent with the following cascading events. As a result of its high reactivity with organic molecules and its low water solubility, O₃ on inhalation penetrates into the lower respiratory tract where it reacts rapidly with components of ALF. Initially, O₃ reacts with antioxidants such as ascorbic acid, reduced glutathione, and uric acid that are contained in the ALF and act as a defense mechanism against oxidative damage by scavenging free radicals and O₃ (3, 7). However, O₃ exposure of sufficient duration and concentration can overwhelm these antioxidants, allowing oxidative damage to occur to airway epithelial cells. We propose that this dose of O₃ is approximated by the cumulative inhaled dose of O₃ at onset of tachypnea.

Once the cumulative inhaled dose of O₃ at onset of tachypnea is reached, the reaction between O₃ and lipid components of the ALF (fatty acids and surfactant) occurs, producing lipid ozonation products in the form of peroxides and aldehydes that then irritate and/or injure airway epithelial cells (8, 11, 23, 24, 32). Derivatives of ozonized phosphatidylcholine (POPC), the predominant phospholipid found in lung lavage fluid, are capable of activating both cytoplasmic phospholipase-A₂ and phospholipase-C (16, 34), which in turn are capable of increasing the availability of arachidonic acid for metabolism, thus producing inflammatory mediators via the cyclooxygenase and lipooxygenase pathways (15, 16). The observation that indomethacin, a cyclooxygenase inhibitor, attenuates O₃-induced rapid shallow breathing and decrements in inspiratory capacity but does not affect the cumulative inhaled dose of O₃ at onset of tachypnea is consistent with this cascade of events. The intrinsic factors that would determine the magnitude of rapid shallow breathing and decrements in inspiratory capacity would be the amount of LOPs produced, their effect on arachidonic acid release, the activation of cyclooxygenase, and the gain of the neural reflex arcs that result in O₃-induced responses (6, 17, 28). Coleridge et al. (17) observed that O₃ inhalation in dogs activated both bronchial C fibers and rapidly adapting pulmonary stretch receptors. In turn, lung C fibers have been shown to be the sensory fibers primarily responsible for O₃-induced rapid shallow breathing in rats (30) and dogs (28). In humans, neural mechanisms have been implicated in O₃-induced symptoms of breathing discomfort (22, 29), decreased inspiratory capacity (13, 22), and rapid shallow breathing (22, 29), as well as bronchoconstriction (4) and airway hyperresponsiveness (12).

The importance of cyclooxygenase product release in the cascade described above is supported by the attenuating effects of indomethacin reported here and elsewhere (26) and the findings of McDonnell et al. (18) who obtained a positive correlation between O₃-induced pulmonary function decrements and the level of prostaglandin E₂ in bronchoalveolar lavage fluid collected within 1 h after the end of exposure in human subjects who varied greatly in ozone responsiveness. The release of cyclooxygenase products of arachidonic acid from injured airway epithelium can thus be viewed as a link in a cascade of events, which begins with the initial reaction of O₃ with the ALF and ends with the observed pulmonary function responses.

Another advantage of the approach used in the present analysis is that by calculating the number of breaths at onset of tachypnea it is possible to better evaluate how breathing pattern and airway geometry affect the onset of O₃-induced responses. The factors that determine the absorption of O₃ into the ALF, as well as the regional distribution of O₃ in the conducting airways during a single breath multiplied by fB, determine the true dose rate of O₃ and time course and magnitude of O₃-induced responses. During each breath, the fractional absorption of O₃ remains relatively constant within a given individual over a twofold change in concentration, VT, or time; however, it varies between individual subjects, ranging from 0.80 to 0.91 when these variables are held constant (25). Increasing VT increases the amount of O₃ absorbed into the ALF, and as a result fewer number of breaths are required to obtain a given dose. In addition, increasing VT will increase the ratio of VT to anatomical deadspace volume and increase the proportion of the total O₃ dose absorbed in the distal conducting airways (5). The increased dose of O₃ being absorbed in the distal conducting airways along with our previous observation (29) that O₃-induced rapid shallow breathing and reduced inspiratory ca-
pacity is initiated by sensory nerves contained within distal conducting airways may account for the trend for cumulative inhaled dose of O₃ at onset of tachypnea to decrease as V⁰ (and as a result V⁰r) increases. In addition, the inclusion of body weight or body surface area in the stepwise regression models for the cumulative inhaled dose of O₃ at onset of tachypnea is consistent with some dimensional component that is proportional to body size, possibly deadspace volume, contributing to individual differences that determine the dose of O₃ needed to induce tachypnea. Further studies need to be conducted in which the deadspace volume, O₃ uptake, and time course of O₃-induced increases in F⁰ are measured and V⁰ is changed while maintaining O₃ concentration to definitively address this issue.

The time course analysis of O₃-induced rapid shallow breathing in human subjects provides a useful tool to better define the chain of events that lead to O₃-induced functional responses, including factors that influence 1) O₃ delivery to the tissue (i.e., the inhaled concentration, F⁰B, V⁰t, and airway geometry); 2) reaction of O₃ with antioxidants contained in the ALF; 3) lipid ozonation products inducing local tissue responses, including cyclooxygenase activation; and 4) stimulation of neural afferents and the resulting reflex responses.

ACKNOWLEDGMENTS

The authors thank Emilie Roy for dedication in reviewing all the raw data files, transcribing by hand the data that was available, and performing the initial data analysis. The authors thank Dr. Michelle Fancchi for reading the revised manuscript and providing editorial comments.

GRANTS

This research was funded by California Air Resources Board Contract A4-070-33 and unrestricted gifts from the American Petroleum Institute and Ventaira Pharmaceutical.

REFERENCES
