Ozone uptake in the intact human respiratory tract: relationship between inhaled dose and actual dose

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Rigas, Marc L., Sandra N. Catlin, Abdellaziz Ben-Jebria, and James S. Ultman. Ozone uptake in the intact human respiratory tract: relationship between inhaled dose and actual dose. J Appl Physiol 88: 2015–2022, 2000.—Inhaled concentration (C), minute volume (MV), and exposure duration (T) are factors that may affect the uptake of ozone (O3) within the respiratory tract. Ten healthy adult nonsmokers participated in four sessions, inhaling 0.2 or 0.4 ppm O3 through an oral mask while exercising continuously to elicit a MV of 20 l/min for 60 min or 40 l/min for 30 min. In each session, fractional absorption (FA) was determined on a breath-by-breath basis as the ratio of O3 uptake to the inhaled O3 dose. The mean ± SD value of FA for all breaths was 0.86 ± 0.06. Although C, MV, and T all had statistically significant effects on FA (P < 0.0001, P = 0.004, and P = 0.026, respectively), the magnitudes of these effects were small compared with intersubject variability. For an average subject, a 0.05 change in FA would require that C change by 1.3 ppm, MV change by 46 l/min, or T change by 1.7 h. It is concluded that inhaled dose is a reasonable surrogate for the actual dose delivered to a particular subject during O3 exposures of <2 h, but it is not a reasonable surrogate when comparisons are made between individuals.

METHODS

Subject characteristics. Ten healthy adult nonsmokers were recruited for this study. Prospective subjects underwent a medical screening, including completion of a medical history, physical examination, and forced expiratory spirometry test to determine forced vital capacity (FVC) and forced expired volume in 1 s (FEV1). Only those individuals who exhibited a FEV1-to-FVC ratio >75% of the predicted value (6) were included in the study.

Subjects were excluded from the study if they reported a history of allergies or of any chronic disease, including asthma, allergic rhinitis, and chronic bronchitis. Subjects were also excluded if they had smoked or been exposed to urban air pollution regularly within 3 yr of the screening. Subjects who reported a respiratory illness or the use of medication (not including contraceptives or vitamin supplements) within 2 wk of a scheduled experimental session were rescheduled at a later date. Female subjects were excluded from further study if they reported that they were pregnant or if positive results were obtained for a hCG pregnancy test administered immediately before each exposure session.

During the screening session, subjects were given an exercise tolerance test on a cycle ergometer. Both minute ventilation and heart rate were monitored as workload was
increased in a stepwise fashion to the subject's maximal level. Each subject was also given a multibreath nitrogen washout test to determine residual lung volume (RV). Total lung capacity (TLC) was estimated by the sum of FVC and RV. All subjects gave informed consent to the screening procedures and the experimental protocol as approved by the Pennsylvania State University Institutional Review Board.

The final group of five men (subjects 1–5) and five women (subjects 6–10) were 18–35 yr old and had the following mean ± SD characteristics: height = 175 ± 13 cm, weight = 71.6 ± 13.4 kg, FVC = 4.46 ± 1.39 liters, RV = 1.62 ± 0.36 liters, and TLC = 6.11 ± 1.57 liters.

Exposure system. Ozonated air produced by a commercial O₃ generator (model 03V1, OREC, Phoenix, AZ) was diluted with room air supplied by a 3.5-hp blower (Craftsman Canister vacuum cleaner, Sears, Chicago, IL) by passing the two streams through a stainless steel in-line mixer (FMX8413T, Omega Engineering, Stamford, CT). The mixed stream, flowing at 200 l/min, entered the top of a 30-liter polycarbonate reservoir and exited through two 1-in.-diameter respiratory hoses at the bottom of the reservoir. One of these exit hoses was connected to the inlet port of the subject's breathing assembly. The other exit hose was vented to a roof exhaust that continuously removed ozonated air that was in excess of the subject's respiratory demand while maintaining near atmospheric pressure inside the reservoir. The O₃ concentration in the reservoir was displayed on a commercial photometric ozone analyzer (1003-AH, Dasibi Environmental, Glendale, CA) that was used to check the calibration of a respiratory O₃ analyzer and to guide adjustment of the ozonation system.

Subjects breathed through a low-dead-volume silicone rubber face mask containing a septum that isolated the mouth and sealed the nose (Series 7900, Hans-Rudolph, Kansas City, MO). As shown in Fig. 1, one end of a pneumotachometer (no. 2, Fleisch, Lausanne, Switzerland) was attached to the oral section of the mask, and the other end was attached to the common port of a two-way nonrebreathing valve (model 2700, Hans Rudolph). Subjects inhaled ozonated air from the 30-liter reservoir through the inlet port of the nonrebreathing valve and exhaled into the room through the outlet port of the valve. The mask-pneumotachometer-valve assembly was supported on the head with an adjustable harness (Hans-Rudolph) that gave the subject adequate mobility during the required exercise.

The inlet sampling line of a custom-built respiratory O₃ analyzer (9) was attached to a 1/8-in.-diameter sampling port located between the exposure mask and the pneumotachometer. When continuously withdrawing 600 ml/min of air from the respired air stream, the analyzer had a linear calibration ($r^2 > 0.99$), a 10–90% step response time of $<90$ ms, a delay time of $\sim300$ ms, and a minimum detectable limit of 0.006 ppm O₃. The analyzer signal was insensitive to changes in temperature, humidity, and carbon dioxide content of respired air.

The two pressure taps on the pneumotachometer were connected by 20-cm-long, 1/4-in.-diameter plastic tubes to a differential pressure transducer (DP45, Validyne Engineering, Northridge, CA). The voltage outputs from the respiratory O₃ analyzer and the pressure transducer were converted and stored as digital data by a computer-based data acquisition system (DAS-1601, Keithley Metrabyte, Cleveland, OH).

Experimental protocol. At the beginning of each experimental session, the baseline of the respiratory O₃ analyzer was adjusted to zero by sampling clean air. The sensitivity of the analyzer was checked by sampling gas from the ozonated air reservoir, the O₃ concentration of which was displayed on the Dasibi photometric analyzer. The dynamic response of the analyzer was also checked by attaching the sampling line to the common port of a three-way subminiature solenoid valve (model 4–8–900, General Valve, Fairfield, NJ) that had one inlet port connected to the ozonated air reservoir and the other inlet port open to room air. By using a relay control circuit (PIO-8, Keithley Metrabyte), the data acquisition system automatically switched the valve inlet position from ozonated air to room air and then determined 1) the time required for the O₃ signal to begin declining (the delay time) and 2) the time required for the O₃ signal to decline 10% to 90% between its initial and final levels (the step-response time). The sensitivity and baseline of the pneumotachometer system were also checked at the beginning of each session by using a calibrated syringe to deliver a known volume of air in both the inspiratory and expiratory directions.

Each subject participated in the four exposure sessions listed in Table 1. At the beginning of each session, a subject
Table 1. Exposure conditions

<table>
<thead>
<tr>
<th>Session Number</th>
<th>c, ppm</th>
<th>Total Exposure Time, min</th>
<th>mv, l/min</th>
<th>Actual Mean VT, liters</th>
<th>Actual Mean VT breathes/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>30</td>
<td>40</td>
<td>1.40</td>
<td>27.6</td>
</tr>
<tr>
<td>2</td>
<td>0.4</td>
<td>30</td>
<td>40</td>
<td>1.49</td>
<td>28.0</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>60</td>
<td>20</td>
<td>1.05</td>
<td>25.4</td>
</tr>
<tr>
<td>4</td>
<td>0.4</td>
<td>60</td>
<td>20</td>
<td>0.95</td>
<td>25.8</td>
</tr>
</tbody>
</table>

The order of the sessions were randomized from subject to subject. c, Target inhaled concentration; mv, target inhaled minute ventilation; VT, tidal volume; f, frequency.

donned the face mask and began exercising on a cycle ergometer (Monark 850, Quinton Instruments, Seattle, WA). The subject synchronized pedaling frequency to an audible metronome set at 60 beats/min. With the results of the exercise tolerance test for employed guidance, the torque on the ergometer wheel was adjusted to achieve the desired MV. As soon as the MV of the subject stabilized, the face mask was connected to the ozonated air reservoir, and the data acquisition system was initiated. Thereafter, the O3 analyzer and pneumotachometer outputs were sampled 100 times per second over 30-s intervals that began 5 min into exposure, 5 min later, and then every 10 min until the end of exposure.

Data computations. The frequency (f), inhalation time, exhalation time, and period of the individual breaths obtained within each 30-s sampling interval were identified from the zero crossing points of the pneumotachometer signal. The inhaled and exhaled tidal volumes (VT) for each breath were computed by numerically integrating the pneumotachometer signal over the inhalation and over the exhalation times, respectively. The f of a breath was taken to be the reciprocal of the breath period. Inhaled and exhaled MV were then obtained as the products of inhaled VT with f and exhaled VT with f, respectively. In effect, each of these two alternative MV represents the ventilation that would occur if an identical breath were repeated for 1 min.

Breath-by-breath calculations of O3 retention required that the O3 concentration and respiratory flow signals be properly synchronized. Whereas the flow measurements were assumed to have a negligible delay time, it was necessary to left-shift the time base of the digitized O3 data accounting for the delay time measured for the O3 analyzer. The amount of O3 inspired within an individual breath was determined by numerically integrating the pneumotachometer signal × time-shifted O3 signal during the inhalation time, and the amount of expired O3 was determined by numerically integrating the same quantity during the exhalation time. The fraction of inhaled O3 retained in the respiratory system, FA, was then computed as one minus the exhaled amount of O3 divided by the inhaled amount of O3.

To analyze the breath-by-breath variation of FA, a database of "good" breaths was developed. A good breath was defined as having inspired and expired VT that were within 20% of one another and as having an inspired VT large that the O3 signal reached a plateau during inhalation. For each good breath, the database contained the subject identification, the type of session, the values of f, the inhaled and exhaled VT, the inhaled and exhaled MV, T and the amounts of inhaled and exhaled O3, and the value of C, computed as the average O3 signal near the end of inhalation. The database contained a total of 2,000 breaths.

Statistical analysis. Because multiple measurements were obtained from individuals at different times, we adopted a multivariate approach with structured covariance for individuals to statistically analyze the FA data in terms of the three factors C, MV, and T. The analysis was based on a linear mixed-effects model (7) given by

\[
(FA)_{jk} = (\beta_0 + \eta_j) + (\beta_1 + \eta_{1j})MV_{jk} + (\beta_2 + \eta_{2j})T_{jk} + (\beta_3 + \eta_{3j})C_{jk} + \epsilon_{jk}
\]

where j denotes the subject and k the observation on the subject. This is a type of two-stage model in which the fixed effects (\(\beta_i, i = 0, 3\)) can be considered as the first stage and the random effects (\(\eta_j, i = 0, 3\)) that vary by subject as the second stage. It was assumed that the components of \(\eta_j = (\eta_{0j}, \eta_{1j}, \eta_{2j}, \eta_{3j})^T\) were independent and identically distributed \(N(0, \Delta)\) where \(\Delta = \begin{pmatrix} 1 & 0.5 & 0.4 \ \ 0.5 & 1 & 0.2 \ \ 0.4 & 0.2 & 1 \end{pmatrix}\) and were independent of \(\eta_j\). Because each measurement period was only 30 s, it was reasonable that each observation would be independent because any effect of time would not be noticeable over this interval. Moreover, statistical tests on observations during these intervals showed that autocorrelation was not significant.

The function in the statistical package Splus (MathSoft, Seattle, WA) was used to fit the linear mixed-effects model. The parameters in the variance structure were estimated by first maximizing the marginal likelihood of the residuals obtained by the least-squares fit. The fixed effects were then estimated via maximum likelihood assuming the variance structure to be known, such as in a generalized least-squares procedure. Computational procedures are detailed in Lindstrom and Bates (8). Approximate standard errors for the fixed effects were derived via asymptotic theory (12).

In addition to running the model for MV and C as continuous variables, we used the target values of these variables (Table 1) for each exposure condition and analyzed them as fixed factors. These fixed factors will be referred to as mv and c.

RESULTS

Continuous data recordings. The raw data taken during a typical series of breaths are shown in Fig. 2, in which the vertical dotted lines indicate the beginning of inspiration. Figure 2A is the calibrated output from the O3 analyzer that was shifted to the left by 28 sampling intervals to account for the 280-ms time delay observed at the beginning of the session. Figure 2B is the calibrated signal from the pneumotachometer. In Fig. 2C, the pneumotachometer signal has been numerically integrated to depict respired volume.

The O3 concentration observed near the end of all breaths dropped to zero or even slightly below zero (Fig. 2A). The negative values of the analyzer output that appeared in some breaths were probably due to a slow thermal drift in the electronics of the O3 analyzer. To eliminate the small effect that this artifact could have on the computations of O3 retention, all the O3 data in a particular breath were shifted upward by a constant that forced the end-expired O3 concentration to be zero.

Breathing patterns. Inspired and expired breathing parameters were not the same. The average value of the inhaled MV for all 2,000 breaths in the database was 28.8 ± 0.2 l/min (mean ± SD), whereas the average expired MV was 30.5 ± 0.3 l/min. The average inhaled VT was 1.10 ± 0.01 liters, whereas the average expired VT was 1.17 ± 0.01 liters. The fact that VT and MV were
~7% larger during exhalation than during inhalation was probably due to the heating and humidification of inhaled air within the respiratory system. In carrying out all the statistical analyses that follow, the inspired MV rather than the expired MV was used as a factor. The expired MV was disregarded for two reasons: first, because the pneumotachometer was calibrated with room air, inspiratory flow measurements were more reliable than expiratory flow measurements; and second, because the exhaled O₃ concentration always reached zero well before the expiration ended, the exhaled MV could not have had an important influence on FA.

Table 1 indicates that the MV of 20 and 40 l/min that were targeted by employing light and moderate exercise challenges, respectively, occurred primarily by differences in VT rather than by differences in f. Moreover, an analysis of all 2,000 breaths in the database indicated that MV was clearly associated with VT (Pearson’s correlation coefficient: $r^2 = 0.44$), whereas MV was essentially uncorrelated with f ($r^2 = 0.01$). This result is consistent with previous reports for subjects exercising within their aerobic range (5) and implies that MV was directly proportional to VT and f was relatively constant.

O₃ retention. The values of FA ranged from 0.56 to 0.98 with a mean ± SD of 0.86 ± 0.06 for all 2,000 breaths. Figures 3, 4, and 5 examine how c, mv, and T influenced FA on a subject-to-subject basis. Although it is not clear in Figs. 3 and 5 whether there was a consistent change in FA with c or with T, Fig. 4 illustrates that FA increased with mv for most subjects. By comparing pooled FA data at the different values of c, mv, and total T as well as for the different subjects, Fig. 6 demonstrates that intersubject differences had the most influence on FA, causing a variation of ~10%.

In the statistical analyses, we elected to include all the measurements from the four sessions, even though data was collected for 30 min more in light exercise sessions 3 and 4 than in moderate exercise sessions 1
and 2. In essence, it was assumed that the exercise effect is linear throughout the exposure period. In fact, the model and conclusions were virtually unchanged when the 30 min of extra data were omitted or when data from the 40- to 60-min intervals were compared with data from the 10- to 30-min intervals in sessions 3 and 4. Because subject 1 did not participate in sessions 2 and 3 and subject 8 did not participate in sessions 3 and 4, it was not possible to estimate random effects for these individuals, and their data were not used in the statistical analysis. A total of 1,833 breaths from eight subjects remained in the database.

The fit of the mixed-effects model to the data was significantly better (via Akaike information criterion, Bayesian information criterion, or likelihood ratio criteria) when \( \eta_3 \) was omitted than when this random effect of inhaled concentration was included in the model. After \( \eta_3 \) was excluded, the results of the model applied to the continuous factors indicated that \( MV (P = 0.0011), T (P = 0.004), \) and \( C (P < 0.0001) \) all had fixed effects that were significantly different from zero. The fixed effects were estimated to be \( b_0 = 0.865, b_1 = +0.00110 \text{ min}^{-1}, b_2 = -0.000499 \text{ min}^{-1}, \) and \( b_3 = -0.0385 \text{ ppm}^{-1}. \) In general, the random effects, \( \eta_{1,i} \) and \( \eta_{2,i} \), were an order of magnitude smaller than the corresponding fixed effects, \( b_1 \) and \( b_2. \) The net effect of \( MV \) on \( FA, \) as judged by \( b_1 + \eta_{1,i,} \) was positive in all except one subject. For subject 9, a negative \( b_1 + \eta_{1,9} \)...
value of \(-0.00114\) min/l reduced the significance of the positive overall influence of MV. The net effect of T on FA, as judged by \(\beta_2 + \eta_2\), was negative in all except one subject. For subject 3, the positive \(\beta_2 + \eta_2\) value of \(+0.000269\) min\(^{-1}\) reduced the significance of the negative overall influence of T.

The same mixed-effects model was also applied to the fixed-factor levels, c and mv. Consistent with the analysis employing continuous factors, mv (\(P = 0.005\)) and c (\(P < 0.0001\)) exhibited fixed effects that were significantly different from zero. Unlike the previous analysis, however, T (\(P = 0.15\)) was not significant. The estimates of fixed effects were \(\beta_0 = 0.891\), \(\beta_1 = +0.0134\) min/l, \(\beta_2 = -0.000261\) min\(^{-1}\), and \(\beta_3 = -0.0613\) ppm\(^{-1}\), and random effects were of the same order of magnitude as the corresponding fixed effects. Subject 9 was again the only individual to exhibit a negative value of \(-0.0136\) min/l for \(\beta_1 + \eta_1\), but subjects 2 and 3 had positive estimates for \(\beta_2 + \eta_2\) of \(+0.000165\) and \(+0.000474\) min\(^{-1}\), respectively.

**DISCUSSION**

In this study, the fraction of inhaled O\(_3\) retained in the respiratory system during a single breath was determined by integrating O\(_3\) concentration and respiratory flow data monitored in 10 healthy adult non-smokers who were orally exposed to either 0.2 or 0.4 ppm O\(_3\) for 30 or 60 min while exercising at light or moderate workloads designed to elicit MVs of 20 or 40 l/min. Because expired O\(_3\) concentration reached zero before the end of each breath, there was no O\(_3\) buildup in the gas spaces of the respiratory tract, and (inhaled dose) FA was equivalent to the amount of O\(_3\) absorbed into the epithelial lining fluid during the course of a breath. Because O\(_3\) rapidly reacts in this protective...
fluid layer, the amount of \( \text{O}_3 \) that reaches the underlying respiratory tract tissue is less than (inhaled dose)·FA. Even so, the measurement and analysis of FA bring us one step closer to knowing tissue dose than the measurement of inhaled dose alone.

The statistical analyses carried out in this study indicated that MV, T, and C all had significant effects on FA. The estimates of the fixed effects, however, suggested that the influence of MV, T, and C may not always be important from a practical point of view. For example, \( \beta_3 \) was estimated as \(-0.0385 \text{ ppm}^{-1}\), so C would have to change by 0.05/0.0385 = 1.3 ppm to induce a 5% change in FA. Because changes in C of this magnitude are highly unlikely in occupational as well as recreational settings, the influence of C on FA is clearly unimportant. By similar reasoning, MV would have to undergo a change of 46 l/min for FA to change by 5%. This might happen if an individual drastically changed his or her level of physical activity between rest and heavy exercise. On the other hand, a change in T of only 100 min would be required to change FA by 5%. This condition is often met in both natural and controlled laboratory exposures.

The FA predicted from the statistical model using MV, T, and C as factors were highly correlated with the FA predicted from the model using MV, T, and C as factors \((r^2 = 0.957)\). This suggests that intersubject variability contributed more to the variability in FA than the inaccuracy of approximating MV and C by their target levels. Figure 6 further shows that differences in FA among subjects were much greater than FA differences caused by changes in MV, C, and T. This may explain why, in previous \( \text{O}_3 \) exposure studies that used inhaled dose or inhaled dose rate as surrogates for actual uptake, the response data from individual subjects were so scattered in the dose-response plots (e.g., Ref. 11).

The positive relationship between FA and MV revealed by the statistical analyses was probably a consequence of the fact that increases in MV were accomplished more as a result of an increase in \( V_r \) than an increase in f. An increase in \( V_r \) implies that \( \text{O}_3 \) penetrates deeper into the respiratory tract, in which absorption is a more efficient process (4). The finding that FA decreased as T increased may have been due to a progressive depletion of reactive substrates in the liquid lining layer (2). This would result in a buildup of \( \text{O}_3 \) "backpressure" that reduced the concentration driving force for absorption.

Only a few measurements of overall respiratory \( \text{O}_3 \) uptake have previously been reported. Employing a pneumotachometer and a rapidly-responding chemiluminescent \( \text{O}_3 \) analyzer similar to the instrument used in the present study, Gerrity et al. (3) determined FA using essentially the same breath-by-breath \( \text{O}_3 \) retention calculations as those employed in the present research. For 10 healthy adults engaged in quiet oral breathing at a C of 0.4 ppm \( \text{O}_3 \), T of \( \sim 60 \) min, and an average MV of 9.6 l/min, the average \( \pm SE \) value of FA was reported to be 0.907 \( \pm 0.010 \). This compares favorably to the average \( \pm SD \) value of 0.86 \( \pm 0.06 \) for the 2,000 breaths analyzed in the present study.

Wester et al. (14) measured average uptake into the lungs of 10 healthy men who breathed quietly from a mask affixed to a large-diameter pipe through which 0.3 ppm ozonated air was supplied at a flow of 40 l/min. A mixing chamber located downstream of the subject was used to dampen fluctuations in \( \text{O}_3 \) concentration. With this apparatus, \( \text{O}_3 \) uptake could be determined by multiplying the steady upstream-to-downstream decrease in \( \text{O}_3 \) concentration with the ozonated air flow entering the pipe. This experimental design avoided the need for a fast-responding instrument so that a slowly-responding commercial \( \text{O}_3 \) analyzer could be utilized. For quiet oral breathing at a C of 0.3 ppm \( \text{O}_3 \), a total T of \( \sim 30 \) min, and an average MV of 10.4 l/min, the average value of FA was reported to be 0.765. This is less than the average value of 0.850 predicted for these breathing conditions by using the fixed-effect estimates of the mixed-effects model.

Another discrepancy is the conclusion by Wester and associates (14) that FA was a decreasing function of MV (their Fig. 4) rather than an increasing function of MV, as found in the present study. Their conclusion was based on FA values that ranged from 0.96 to 0.51 as MV increased from 8 to 12 liters. This is in contrast to the present study in which the FA measured in 2,000 individual breaths ranged from 0.56 to 0.98 as MV increased from 10 to 70 l/min. It is possible that there was a difference in breathing patterns of the subjects in the two studies; the subjects in the present study tended to increase MV by increasing \( V_T \), whereas the subjects in the previous study may have increased f as well as \( V_T \). In all probability, however, the conclusion of Wester and associates was an artifact of a large intersubject variation occurring over a comparatively narrow range of MV. Moreover, the precision of the uptake measurements of Wester and associates is somewhat suspect because they reported up to 25% variability in uptake within one subject between two different laboratory visits. The session-by-session variability in the present study was 6% at most.

In conclusion, when a particular subject is exposed to a fixed concentration of \( \text{O}_3 \) at a fixed exercise level, inhaled dose is a reasonable surrogate for the actual uptake of \( \text{O}_3 \) by the respiratory system, as long as the T is \(< 2 \) h. On the other hand, the actual dose may vary considerably among individuals who are exposed to similar inhaled doses.
REFERENCES


