The effect of exercise on nasal uptake of ozone in healthy human adults

Keegan Sawyer, James S. Brown, Milan J. Hazucha, and William D. Bennett

Center for Environmental Medicine, Asthma, and Lung Biology, University of North Carolina, Chapel Hill, North Carolina

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Sawyer K, Brown JS, Hazucha MJ, Bennett WD. The effect of exercise on nasal uptake of ozone in healthy adult humans. J Appl Physiol 102: 1380–1386, 2007. First published November 2, 2006; doi:10.1152/japplphysiol.00269.2006.—The nose may help protect the lower respiratory tract from the effects of ambient ozone by scrubbing ozone from inspired air. Reductions in both nasal resistance and nitric oxide production with exercise may influence the efficiency of ozone uptake in the nose. Nasal ozone uptake was measured in 10 healthy volunteers before and after 15 min of moderate bicycle exercise. Ozone (0.2 parts/million) was pulled through both nostrils and out of the mouth at a constant flow while the subjects closed their epiglottises. Nasal ozone uptake was determined by comparing the ozone concentration entering the nostrils to that exiting the mouth. Average preexercise uptake of ozone was 56 ± 7.8 and 37 ± 4.9% at 10 and 20 l/min, respectively. These averages did not significantly differ from those immediately postexercise (55 and 37%). Nasal ozone uptake increased significantly (P < 0.001) with decreasing flow rate, but intersubject variability in uptake could not be predicted by nasal volume or cross-sectional areas (as measured by acoustic rhinometry) or endogenous nitric oxide production. However, the percent change in ozone uptake after exercise, within an individual, was correlated with both 1) percent change in nasal volume (r = 0.70 at 10 l/min) and 2) percent change in the rate of volumetric expansion between the nasal valve and turbinates (r = 0.82 at 10 l/min). These results may be useful for assessing human risk associated with ozone exposure during exercise.

ozone dosimetry; nasal breathing; exercise

THE NOSE PROVIDES A DEFENSE for the lungs by partially filtering inhaled pollutant gases and particles before they can reach the lungs. Nasal breathing during exercise has been observed in humans up to maximal minute ventilations of 60 l/min; however, the switch from nasal to oronasal breathing usually occurs at ∼35 l/min (19). In addition, exercise naturally dilates the nasal airways (23) and reduces nasal resistance (Rn) (21). However, the rate, but intersubject variability in uptake could not be predicted by ozone dosimetry; nasal breathing; exercise.

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MATERIALS AND METHODS

Human subjects and screening. This study was approved by the Committee on the Protection of Human Rights of Human Subjects, School of Medicine, University of North Carolina at Chapel Hill. Before participating in this study, all subjects were informed of study-associated risks and signed a statement of informed consent. Recruited subjects were between 18 and 35 yr of age with no history of smoking, lung disease, nasal surgery or obstructions, or regular use of recreational drugs inhaled either orally or nasally. Included subjects had no prolonged prior usage or use within 4 wk of nasal steroids, antihistamines, or decongestants and were free of upper and lower respiratory infections for at least 4 wk before the study. Also, included subjects were able to close their epiglottis for a minimum of 10 s. Fifty percent of screened volunteers could not maintain a closed epiglottis.

We prescreened subjects to determine their maximal exercise capacity on a bicycle ergometer while pedaling at a rate between 60 and 70 rpm. The 15-min prescreening was split into three graded and increasing 5-min submaximal exercise workloads (ranging from 25 to 125 W), concurrently monitoring heart rate with a three-lead ECG. A subject’s maximum physical work capacity was then predicted via linear extrapolation of the exercise workload (Watts) – heart rate relationship to individual predicted maximum heart rate adjusted for age (2, 20). Eight women and two men, recruited from the area in and around Chapel Hill, NC, passed screening to participate in the study.

O₃ uptake measurements. To measure the nasal uptake of O₃, we used a nasal O₃ exposure system (Fig. 1) in which O₃ is pulled through a subject’s nose and out through his/her mouth. Subjects were required to hold their breath and keep their epiglottis closed during the measurement. Normally, closing the epiglottis tends to be an involuntary response that causes the upper airways to be isolated from the lower airways (e.g., before a cough to increase pressure in the thorax). For O₃ uptake in upper airways to be measured, the epiglottis had to be kept closed for a minimum of 10 s to obtain adequate uptake data. If subjects could not perform the breath-holding maneuver required to make the O₃ uptake measurements, they were dismissed from the remainder of the study. Fifty percent of screened subjects were unable to perform this maneuver.

A 0.2 ppm O₃ concentration was generated by a photometric O₃ calibrator (API, model 410 San Diego, CA) and stored in a 60-liter Tedlar bag. Teflon tubing led from the Tedlar bag to a pneumotachograph (Hans Rudolph, Kansas City, MO) and ultimately to hollowed-out nose olives that the subject inserted into each nostril. Subjects clamped their lips around a pear-shaped Teflon mouthpiece, which filled the mouth cavity. Tubing connected the mouthpiece to a flow-regulating valve and then a vacuum source. Flow rate was regulated by a valve between the vacuum pump and the mouthpiece. The actual flow entering the nose was measured by the pneumotachograph connected to a pressure transducer (Validyne Engineering; Northridge, CA). O₃ sampling ports were positioned at the nostrils and mouth. A three-way Teflon valve connected lines from the sampling ports to a fast-acting O₃ analyzer (6). O₃ samples were collected at a rate of 3 ml/s through 22-gauge Teflon tubing. Before each measure of O₃ uptake, we sampled O₃ entering and exiting the mouth for 1–3 s to allow the sampled signal to reach a steady-state value. To avoid O₃ reaction with condensate, the three-way valve was positioned to pull room air through the sample line from the mouth port to the O₃ analyzer between maneuvers. Both flow and O₃ concentration were acquired at a rate of 20 Hz by a MacLab data acquisition system and saved for subsequent analysis. The nasal uptake of O₃ was calculated as:

\[
\text{Uptake} = 1 - \left( \frac{[O_3]\text{ exiting the mouth}}{[O_3]\text{ entering the nose}} \right)
\]

AR. AR (Hood Laboratories, Pembroke, MA) was used to assess nasal cavity geometry. For this technique, sound pulses are emitted from a wave tube into the nasal cavity via a nose tip inserted a few millimeters inside a nostril. The nose tip of the wave tube is coated with vaseline to ensure a good acoustic seal. A microphone within the wave tube recaptures the incident and reflected sound waves from the nasal cavity. EcoVision Software converts the collected wave signals into a rhinograph (Fig. 2) depicting nasal cavity cross-sectional area as a function of distance from the wave tube tip. Most rhinographs show three distinct “valleys” referred to as the MCA, cross-sectional area two (CSA2), and cross-sectional area 3 (CSA3). These cross-sectional areas (i.e., the MCA, CSA2, and CSA3) correspond to the nasal valve, the anterior edge of the nasal turbinates, and the posterior edge of turbinate respectively (14). The nasal valve, the narrowest cross-sectional area, is the major “resistive segment” within the nasal passageway (4) and dictates the flow condition of inhaled air (27). The EcoVision software also calculates Vn and resistance values between selected points within the rhinograph (9). For any given AR assessment, we performed two measurements on each subject’s right and
left nostrils. Cross-sectional area, Vn, and Rn from each of the four measurements were averaged.

NO measurement. Nasal NO concentration was measured with NO chemiluminescence analyzer (Siever, model 270B, Boulder, CO). A sample line was connected from the NO analyzer to one nostril via a nasal olive, while the other nostril was open to ambient air. NO is undiluted by air from the nasopharynx. NO was repeatedly measured from both the right and left nostrils of each subject. NO production, in nanoliters of NO per minute, was calculated by multiplying NO concentration (parts/billion) by the sampling rate (0.5 l/min).

Study design. Fractional O3 uptake measurements were conducted on subjects before and after moderate exercise. Nasal O3 uptake measurements were repeated three times at each of two flow rates (10 and 20 l/min). Before and immediately after the O3 uptake measurements, AR and nasal NO measurements were completed. Subjects exercised 15 min at between 40 and 60% of their maximum physical work capacity (determined during screening) on a bicycle ergometer. The AR and NO measurements together took ~3 min to complete. Nasal O3 uptake measurements were then repeated, again three measurements at both flows. The time between each measurement was recorded to ensure constancy between subjects. Immediately following the O3 uptake measurements, AR and NO measurements were repeated to ensure that neither the O3 exposure during the uptake measurement or the time required to make the measurements caused changes in nasal geometry or NO production. Table 1 illustrates this sequence of measurement events.

Data analysis. The statistical package SPSS (version 10) was used to conduct paired t-tests to 1) analyze O3 uptake, NO, and AR values before and after exercise, and 2) determine whether NO and AR values were affected by the O3 uptake measurements or temporal biases. Linear regression analysis was conducted to examine AR parameters and NO production as predictors of O3 uptake.

RESULTS

Intersubject variation in mean O3 uptake ranged from 26.8 to 64.8% (preexercise) and 27.2 and 65.4% (postexercise). The intrasubject variability for each set of triplicate uptake measurements was low. The coefficient of variation was always <10% at any flow condition pre- or postexercise. The results of the paired t-tests for mean nasal uptake of O3 are given in Table 2. There was a significant difference in O3 uptake between the two flow rates 10 and 20 l/min both pre- and postexercise (Fig. 3). The percent difference (±SD) in O3 uptake at the 10 l/min flow was nearly 50% greater than that at the 20 l/min flow rate (51.8 ± 9.7 and 48.9 ± 10.3 pre- and postexercise, respect-

Table 1. Visit schedule for study participants

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Procedure</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>Preexercise, pre-ozone uptake measurements</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>of NO and AR</td>
<td></td>
</tr>
<tr>
<td>6–16</td>
<td>Preexercise ozone uptake</td>
<td></td>
</tr>
<tr>
<td>17–22</td>
<td>Preexercise, post-ozone uptake measurements</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>of NO and AR</td>
<td></td>
</tr>
<tr>
<td>23–38</td>
<td>Bicycle exercise</td>
<td></td>
</tr>
<tr>
<td>39–41</td>
<td>Postexercise, pre-ozone uptake measurements</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>of NO and AR</td>
<td></td>
</tr>
<tr>
<td>41–46</td>
<td>Postexercise ozone uptake</td>
<td></td>
</tr>
<tr>
<td>47–50</td>
<td>Postexercise, post-ozone uptake measurements of NO and AR</td>
<td>D</td>
</tr>
</tbody>
</table>

Groups A and C were used to test for exercise effect on acoustic rhinometry (AR) and nitric oxide (NO) results. C and D to test for a postexercise time lapse effect on AR and NO results, and A and B to test the effect of O3 exposure during the uptake measurements on AR and NO parameters.

Fig. 2. Illustration of rhinograph. The dashed line represents the end of the wave tube tip. MCA, CSA2, and CSA3 correspond to the nasal valve, the anterior edge of the nasal turbinates, and the posterior edge of turbinates, respectively.

Fig. 3. Exercise and flow effects on nasal O3 uptake (bars indicate standard error).

Table 2. Comparison of nasal ozone uptake pre- and postexercise at 10 and 20 l/min

<table>
<thead>
<tr>
<th>Pairs</th>
<th>Ozone Uptake, %</th>
<th>Correlation (r) Between Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre 10 l/min</td>
<td>55.7 ± 7.82</td>
<td>0.88</td>
</tr>
<tr>
<td>Pre 20 l/min</td>
<td>36.7 ± 4.93*</td>
<td></td>
</tr>
<tr>
<td>Post 10 l/min</td>
<td>54.6 ± 7.97</td>
<td>0.89</td>
</tr>
<tr>
<td>Post 20 l/min</td>
<td>36.8 ± 5.53*</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significantly different (P = 0.001) from uptake at 10 l/min.
Exercise did not have a significant effect on O3 uptake at either flow rate.

To determine the approximate amount of O3 delivered to the lung (i.e., after passing through the nose), a dose rate was calculated.

Five nasal parameters (Vn, Rn, MCA, CSA2, CSA3) and NO production were compared pre- and postexercise. Vn and Rn were determined for the area that extends between MCA and CSA3. There were statistically significant (P = 0.05) increases in Vn, MCA, CSA2, and CSA3 post- vs. preexercise (Fig. 4). Rn decreased 35% post- relative to preexercise (P < 0.01). There was a 10.6% decrease in nasal NO production postexercise, but this finding was not statistically significant.

Linear regression analysis was used to examine the dependence of nasal O3 uptake on Vn, Rn, NO production, and the CSA2-to-MCA ratio. The CSA2-to-MCA ratio was thought to represent the rapid changes in flow velocity and associated flow separation that may occur distal to the nasal valve. None of these parameters were found to be significant predictors of nasal O3 uptake either pre- or postexercise. The O3 exposure that occurred during the uptake measurements did not significantly affect Vn, Rn, MCA, CSA2, or NO production (Table 3).

The percent change in nasal O3 uptake (from pre- to post-exercise) was significantly associated with percent changes in nasal Vn and CSA2-to-MCA ratio (see Figs. 5 and 6, respectively). At 10 l/min, there were significant linear relationships between percent change uptake and both percent change in Vn and percent change in CSA2-to-MCA ratio. At 20 l/min, there was also a significant linear relationship between percent change nasal uptake and percent change in Vn but not with percent change in CSA2-to-MCA ratio.

**DISCUSSION**

The aim of this study was to determine the effects of exercise-induced physiological changes in the nasal passages on the O3 uptake in the nose at two different flow rates. Mean nasal uptake of O3 at 10 l/min was ~50% greater than at 20 l/min (P = 0.001). This flow effect is consistent with Santiago et al. (24), who also showed a decreasing fractional uptake as flow rate through the nose increased. Santiago et al. (24) attributed this relationship to a decreased residence time with increasing flow rate, i.e., reducing the time available for O3 to diffuse to the airway walls and chemically react with substrates in the mucus lining layer. Because O3 is also known to react with NO in the nose (20), increasing the O3 delivery rate relative to the steady-state production of NO in the nose might have also contributed to reduced fractional uptake at the higher flow rates. On the other hand, there was no correlation between O3 uptake and nasal NO concentrations across all subjects at any given flow rate, suggesting that, although NO may play a minor role in O3 uptake in the nose, it is likely not the principal determinant for removing O3 from inspired air.

Although the nasal O3 uptake efficiency was 50% greater at the 10 l/min volumetric flow, the inhaled O3 rate (delivered dose) to the lung was greater at the 20 l/min volumetric flow due to the higher minute ventilation. To determine the approximate rate of O3 delivered to the lung (i.e., after passing through the nose), a delivered dose rate was calculated as follows:

\[
\text{Delivered dose rate} = \text{flow rate} \times [\text{O}_3 \text{ ppm}] \times (100 - \% \text{ nasal O}_3 \text{ uptake})
\]

Preexercise the delivered dose rate to the lungs was 0.89 ppm·l⁻¹·min⁻¹ and 2.53 ppm·l⁻¹·min⁻¹ at 10 and 20 l/min respectively. Postexercise, the delivered dose rate at 10 l/min was 0.91 ppm·l⁻¹·min⁻¹ and 2.53 ppm·l⁻¹·min⁻¹ at the 20 l/min flow rate. Both pre- and postexercise, the delivered dose rate at the high flow rate (20 l/min) was ~1.6 times higher than the delivered dose rate at the low flow rate (10 l/min).

Similar to Santiago et al. (24), we found no significant intersubject relationship between Vn and nasal uptake of O3 at either 10 or 20 l/min. In theory, O3 uptake should be proportional to the transit time through a defined airspace. Thus increasing Vn should increase the transit time and ultimately increase O3 uptake. There are several plausible reasons why this relationship was not observed. First, airflow through the nasal cavity does not encompass the entire volume of the nasal cavity (4, 27). In fact, airflow seldom reaches many parts of the nasal cavity. As a result, measurements on the total increase in nasal Vn may not truly reflect the relative increase in volume along the main airflow passageways. Total nasal Vn, therefore, may be an insensitive parameter by which to predict percent O3 uptake. Second, we only observed relatively small changes in nasal Vn with exercise. On average, we observed a 30% increase in Vn following exercise. With greater nasal dilatation (i.e., greater changes in percent volume), there may have been a more readily observable volume effect on O3 uptake. On the other hand, even with heavy exercise, others have only observed a 50% reduction in Rn (26) compared with the 35% we observed here. There is certainly a maximum dilatory capacity

| Table 3. Pairwise comparison of nasal parameters pre- and post-ozone uptake |
|-----------------|-----------------|-----------------|-------|
| Nasal Parameter | Pre-Ozone       | Post-Ozone      | P Value |
| Vn, cm³         | 7.65±0.72       | 7.08±1.13       | 0.26   |
| Rn, cm³·H₂O·l⁻¹·min⁻¹ | 0.98±0.40       | 1.08±0.51       | 0.52   |
| MCA, cm²        | 0.47±0.10       | 0.45±0.11       | 0.45   |
| CSA2, cm²       | 0.92±0.11       | 0.84±0.10       | 0.14   |
| NO, nl/min      | 333±167         | 319±148         | 0.10   |

Values are means ± SD. Vn, nasal volume; Rn, nasal resistance; MCA, minimum cross-sectional area; CSA2, cross-sectional area 2; NO, nitric oxide. Values are preexercise. See text for definition of parameters.
for the nose that we were already approaching in these healthy subjects.

To explore the possibility that those with the greatest exercise-induced nasal dilation would have the greatest change in O₃ uptake, we plotted the percent change in Vn vs. the percent change in nasal O₃ uptake at each flow rate (Fig. 5). Strong correlations between percent change in Vn and percent change in O₃ uptake were observed at both flow rates. Despite the strong correlation, the overall effect is small. The highest percent change in volume, 71%, effected no greater than a 20% and 6% increase in nasal O₃ uptake at the 20 and 10 l/min flows, respectively. It should be noted that this 71% increase in volume was by far the largest we observed. The relationship between percent change in volume and percent change in uptake is not significant when these values are excluded. Also important, many percent changes in O₃ uptake were negative at both flow rates despite increases in Vn (Fig. 5). This implies that other factors tend to reduce nasal O₃ uptake following exercise. For example, other studies report statistically significant reductions in NO following exercise that could contribute to reduced O₃ uptake (15, 22). Although we observed a tendency for reduced NO production following exercise, these changes were not correlated with the changes in O₃ uptake. Finally, there may have been other effects of exercise on nasal physiology that were not measured in our study. As discussed by Santiago et al. (24), the airways produce antioxidant species like uric acid and glutathione that readily react with O₃. Exercise-induced reductions in the airway concentrations of these components may counteract the effect of increased nasal Vn on nasal O₃ uptake.

Our study augments knowledge obtained from previous studies by also investigating the relationship between exercise-induced changes in Rn, MCA, CSA2, and nasal NO production on nasal O₃ uptake. Pairwise comparisons of these values before and after exercise demonstrated significant changes among all parameters except for nasal NO production (see Fig.

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**Fig. 5.** Linear regression of change in O₃ uptake vs. change in nasal volume due to exercise. Dashed line, 10 l/min; solid line, 20 l/min.

**Fig. 6.** Linear regression of change in O₃ uptake vs. change in CSA2-to-MCA ratio due to exercise. Dashed line, 10 l/min; solid line, 20 l/min.
4). Despite these changes, there were no significant relationships between nasal $O_3$ uptake and any of these parameters, nor was there a net effect of exercise on $O_3$ uptake. A statistically significant relationship emerged between changes in $O_3$ uptake and changes in CSA2-to-MCA ratio (Fig. 6). Similar to the analysis of changes in nasal $V_n$ (Fig. 5), changes in CSA2-to-MCA ratio affected small (<20%) changes in $O_3$ uptake. However, both relationships may be driven by gender differences, since individuals with the largest changes in $V_n$ and CSA2-to-MCA ratios are men. When the male subjects are dropped from the analysis, the relationship disappears.

To verify that $O_3$ exposure during the uptake measurement did not affect AR endpoints, pairwise comparisons were made between the preexercise pre-$O_3$ AR parameters and the preexercise post-$O_3$ AR parameters (Table 3). There was no statistically significant effect of the $O_3$ uptake measurements on any of the AR parameters. In addition, to make certain nasal dilation associated with exercise (determined by $V_n$ and AR parameters) did not diminish over time (i.e., time during which postexercise $O_3$ uptake measurements were being made), comparisons were made between the postexercise pre-$O_3$ data and the postexercise post-$O_3$ data. Again, no statistically significant differences in AR parameters were observed.

The measurement technique, breath holding by closing the epiglottis, was too difficult for 50% of the screened subjects. Some subjects were not able to sustain a closed epiglottis for longer than a few seconds. Other subjects were possibly closing their soft palate while trying to close the epiglottis. The soft palate closes off the nasal passageway from the oral cavity, whereas the epiglottis separates the upper and lower airways. To determine whether selection bias affected our results, an analysis of $R_{ns}$ between the selected and dismissed subjects was conducted. No significant differences were observed to indicate a selection bias.

Other investigators also measured nasal $O_3$ uptake efficiency (6, 24). We previously discussed the similarities between our findings and Santiago et al. (24). Gerrity et al. (6) investigated nasal vs. oral $O_3$ uptake in 18 healthy adult men between the ages of 18 and 35. Subjects were exposed to three $O_3$ concentrations (0.1, 0.2, and 0.4 ppm) within an environmental chamber. Subjects breathed in one of three manners (nose only, oronasal, and nasal exposures). Hynes et al. (11) exposed healthy young adults for 30 min during moderate continuous exercise. Adams et al. (1) exposed healthy young men during moderate and heavy continuous exercise for oral vs. oronasal breathing. In these two studies, the researchers found no flow-dependent change in $O_3$ uptake as they increased flow between 21 and 38 l/min inspiratory flow, respectively. A pediatric feeding tube ran through one nostril into the posterior pharynx to sample airway $O_3$ concentration. Unlike Santiago et al. (24), Gerrity et al. (6) found no concentration dependence in $O_3$ uptake efficiency, although uptake tended to decrease with increasing concentration. On the other hand, Gerrity et al. (6) observed a significant difference ($P < 0.001$) in uptake at the two different breathing rates, in agreement with both the Santiago et al. study (24) as well as our current study. However, uptake at 24 breaths/min was only 6% less than at 12 breaths/min, a difference smaller than the difference in uptake observed by Santiago et al. (24) and our study.

To compare our quantitative measures of $O_3$ uptake with those of others (6, 24) is somewhat difficult since we each used different methodologies. Santiago et al. (24) pulled $O_3$ through one nostril and out through the second nostril while the soft palate was closed, whereas we pulled $O_3$ through both nostrils and out the mouth. Santiago et al. (24) assumed that respiratory flow through one nostril would be equivalent to half the total respiratory flow going through both nostrils. There is not clear evidence that total flow rate is split evenly between nostrils during respiration. The presence of the nasal cycle, in fact, suggests this is not the case. It is also difficult to determine how much of the measured uptake is due to flow in the inspiratory vs. expiratory direction for their method. Likewise, our method of drawing the $O_3$ in the nose and out the mouth is confounded by the inability to distinguish the influence of nasal inspiratory vs. oral expiratory uptake. For our purposes, however, distinguishing the different routes of uptake was not necessary to assess the nasal changes induced by exercise. If we ignore as negligible the expiratory efficiency through the single nasal passageway (24) and the expiratory efficiency through the oral cavity (current study), then $O_3$ uptake at 5 l/min for Santiago et al. (24) was 65% compared with our mean 56% at 10 l/min (assuming 5 l/min through each nostril). Gerrity et al. (6) measured uptake under spontaneous breathing conditions and was better able to isolate inspiratory extrathoracic uptake by sampling in the posterior pharynx. Their inspiratory flow rates were generally higher than those used by us or Santiago et al. (24). The mean flow rate in Gerrity et al. (6) was 27 l/min, whereas our highest flow rate was 20 l/min. Under these conditions, Gerrity et al. (6) found extrathoracic uptake by nasal breathing to be 36%, which compares to our mean uptake of 37% at 20 l/min (again assuming the expiratory oral cavity uptake is negligible in our case). The fact that Gerrity et al. (6) found no flow-dependent change in $O_3$ uptake as they increased flow between 21 and 38 l/min suggests that the efficiency of the upper airways for removing $O_3$ approached a minimum. Although consideration of these absolute measures of uptake may be useful for dosimetry modeling, care should be taken to consider the conditions and methodologies by which they were determined.

A few investigators (1, 11) compared $O_3$ health effects associated with different routes of breathing. Adams et al. (1) and Hynes et al. (11) both investigated FEV$_1$ response to 0.4 ppm $O_3$, comparing the difference in FEV$_1$ response from oral, oronasal, and nasal exposures. Hynes et al. (11) exposed healthy young adults for 30 min during moderate continuous exercise. Adams et al. (1) exposed healthy young men during moderate and heavy continuous exercise for oral vs. oronasal breathing. In these two studies, the researchers found no significant difference in FEV$_1$ response to 0.4 ppm $O_3$ exposure based on route of exposure. Results from Adams et al. (1) and Hynes et al. (11) suggest that there is little difference in $O_3$ uptake efficiency between the oral and nasal respiration routes, consistent with findings by Gerrity et al. (6). On the other hand, Kabel et al. (13), comparing uptake of serial inhaled $O_3$ boluses for oral vs. nasal breathing, found that the nasal passages are 60% more efficient at removing $O_3$ than the oral pathway. Furthermore, Kabel et al. (13) found that $O_3$ penetrated deeper into the lung during oral breathing than during nasal breathing, suggesting that the distal lung may be more susceptible to $O_3$ toxicity under oral breathing conditions. It is important to emphasize that the findings of Adams et al. (1) and Hynes et al. (11) are specific to changes in FEV$_1$, an acute response to $O_3$ exposure. Differences in oral vs. nasal $O_3$ uptake may be more important for acute inflammatory end points, for asthmatics with airway hyperresponsiveness to $O_3$, or for chronic lung effects associated with $O_3$ exposure.
An area for future study, suggested from our results, is gender differences in nasal O₃ uptake. We studied eight women and two men. The men exhibited large exercise-induced changes in nasal Vn (71.5 and 52.2%) and CSA₂-to-MCA ratio (41.5 and 32.9%). These changes, when plotted against percent change in O₃ uptake, stand out from the women’s data (see Figs. 5 and 6). Three potential research questions arise from this: 1) Are there gender differences in baseline and exercise induced change in nasal O₃ uptake? 2) If there is a gender difference, is it caused by differences in nasal physiology? 3) If there is a gender difference, does it result in differential health risk from O₃ exposure? The third question has been addressed. Gender does not appear to affect O₃ sensitivity (17), and physiological responses of young healthy women to O₃ exposure appear comparable to the response of young men (8).

In conclusion, this study addressed 1) the effect of moderate exercise on the uptake of O₃ in the nose and 2) nasal physiological predictors of nasal O₃ uptake. In agreement with previous studies, nasal uptake of O₃ increased with decreasing flow rate. Since flow rate increases with exercise, higher O₃ logical predictors of nasal O₃ uptake. In agreement with previous studies, nasal uptake of O₃ increased with decreasing flow rate. Since flow rate increases with exercise, higher O₃ concentrations are introduced to the lower respiratory tract. Physiological changes induced by moderate exercise did not have a dramatic effect on nasal O₃ uptake. This may be caused by unmeasured exercise-induced physiological or biochemical changes that offset the impact of the parameters measured in this study.

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Current address of J. S. Brown: National Center for Environmental Assessment (B243-01), US EPA, RTP, NC 27711.

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REFERENCES