Abstract

Exercise is known to reduce nasal resistance and nitric oxide production. These changes may influence nasal uptake of ozone. Nasal ozone uptake was measured in ten healthy volunteers before and after 15 minutes of moderate, bicycle exercise. A nasal exposure system was used in which a constant airflow and constant ozone concentration (0.2 ppm) was pulled through both nostrils and out of the mouth while the subject closed their epiglottis. Nasal uptake, as a percent of ozone entering the nose, was determined by comparing a sample of ozone concentration entering the nostrils to a sample of ozone concentration exiting the mouth. Average pre-exercise uptake of ozone was 56% (± 7.8) and 37% (± 4.9) at 10 and 20 l/min respectively. These averages did not differ significantly from those immediately post-exercise (55% and 37%). Nasal ozone uptake increased significantly (p < 0.001) with decreasing flow rate, but intersubject variability in nasal uptake could not be predicted by nasal volume, 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 positively correlated with both 1) percent changes in nasal volume (r = 0.70 at 10 l/min) and 2) percent change in the ratio of the second minimum cross-sectional area to the minimum cross-sectional area (r = 0.82 at 10 l/min). These results may be useful for assessing human risk associated with ozone exposure during exercise.
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INTRODUCTION

Health Effects of Ambient Ozone Exposure

Ozone (O₃) is a highly reactive insoluble gas, composed of three bound oxygens. It is present in both the Earths’ stratosphere, as a protective layer against UV radiation, and in the Earth’s troposphere, as a severe atmospheric pollutant. Tropospheric ozone is the primary component of photochemical smog. It is the photochemical reaction product of nitric oxides (NOx) and volatile organic carbons (VOCs) in the presence of heat and light. Most NOx and VOC emissions have an anthropogenic source, particularly motor vehicle exhaust and industrial combustion. Ozone formation and consumption reactions, often referred to as a photolytic cycle, can be represented as follows:

\[ \text{NO}_2 + hv \rightarrow \text{NO} + \text{O} \cdot \]
\[ \text{O} \cdot + \text{O}_2 \rightarrow \text{O}_3 \]
\[ \text{O}_3 + \text{NO} + hv \rightarrow \text{NO}_2 + \text{O}_2 \]

Solar radiation is represented as \( hv \), and the oxygen radical is represented as \( \text{O} \cdot \). Without the presence of atmospheric VOCs, this reaction cycle would reach a steady-state condition. However, the photolysis of VOCs creates the intermediate free radicals that oxidize nitric oxide (a major component of NOx) to nitrogen dioxide (NO₂). This increases the available NO₂ for ozone production. As a result, peak concentrations of tropospheric ozone are directly related to the ratio of atmospheric VOCs to NO₂. Because these reactions require sun light and heat, the highest observed ozone concentrations typically occur during summer months, when heat and light intensity are at their highest.
Control techniques, however, focus on limiting NO₂, VOC, or both emissions. (WHO 2000, Casarett and Doull 1996, US EPA1996)

Ozone exposure can significantly damage the human respiratory system. Due to its reactive nature, ozone readily oxidizes biochemical components in the upper and lower airways. The result is a range of health responses from reversible airway inflammation to permanent lung tissue damage, frequently accompanied by subjective symptoms including cough, wheezing, and chest pain.

A great deal of data from human clinical studies has been compiled on the acute health outcomes associated with ambient ozone exposure. Among the most common of these are decreases in lung volume and increased airway resistance, increased airway reactivity, and increased airway inflammation. Researchers typically include an intermittent (IE) or continuous exercise (CE) protocol in order to mimic real-world activity (ventilation) level. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) are typical spirometric measurements assessing changes in lung function. FVC and FEV1 are obtained by recording maximum total and within one second, respectively, forced exhaled air from lungs immediately after maximum inhalation (filling the lungs). Decreases in these values are indicative of airway obstruction and/or airway restriction. Hazucha et al (1973) were among the first to establish decreased FVC and FEV1 in exercising humans exposed to ozone. In this study, subjects were exposed to 0.35 ppm and 0.75 ppm ozone for two hours with 15 minutes of light exercise on a bicycle ergometer. Significant decreases in FVC and FEV1 were observed at both concentration levels. Two criticisms of this study are: (1) subjects never reached a ventilation rate that matched that of typical outdoor activity ventilation
(Delucia 1977), and (2) 0.35 and 0.75 ppm exposure concentrations are greater than the EPA 0.3 ppm cut-off for typical U.S. "ambient" ozone concentration. However, McDonnell et al. 1983 observed significant FVC and FEV1 decrements at 0.12 ppm in human subjects exposed for 2.5 hours with alternating 15 minutes of moderate treadmill exercise and rest for the first two hours. Further, Horstman et al. (1990) recorded FEV1 decrements at 0.10 and 0.08 ppm in subjects exposed for 6.6 hours with six 50 minute periods of heavy exercise alternating between a bicycle ergometer and a treadmill. In both of these studies, subjects reached ventilation rates consistent with moderate or heavy outdoor activity. Also evident in both studies was a relationship between ozone concentration and lung function. Hazucha et al. (1992) clarifies this relationship by establishing that FEV1 decreases with increasing dose of ozone. On the other hand, research also shows that repeat exposures to ambient ozone concentrations leads to an attenuation of spirometric responses 24 to 48 hours after exposure (Folinsbee 2000 and 1994, Gilner 1983).

Increased airway responsiveness, or airway hyperactivity, is another common acute response to ambient ozone exposure. Airway responsiveness refers to the ability of various environmental stimuli, like ozone or pollen, to induce bronchoconstriction, a condition typically associated with diseases like asthma and chronic bronchitis (EPA 1996). Airway reactivity is determined by measuring FEV1 (or any forced expiratory endpoint) before and immediately after step-wise exposure to a known stimulus, typically methacholine or a type of allergen. Gong et al. (1986) and Folinsbee et al. (1988), challenged healthy subjects pre-exposed to ozone with Histamine and Methacoline, respectively. Gong established greater than twenty percent increase in airway reactivity in
short-term (1 hour) exposure to 0.2ppm, while Folinsbee et al (1988) demonstrated significantly increased airway reactivity in healthy adults exposed to 0.12ppm for 6.6 hours. As with spirometric endpoints, airway reactivity attenuates after repeat exposures (Kulle 1982), although there is not an apparent relationship between forced expiratory decrements and heightened airway reactivity (Folinsbee 1988).

Airway inflammation is a third important health consequence of ozone exposure. Researchers usually measure airway inflammation through bronchoalveolar (BAL) or nasal (NL) lavage techniques. Cell samples are taken from respiratory airways before and after ozone exposure and analyzed for increased levels of inflammatory “markers” such as cytokines (IL-6, IL-8 etc), polymorphonuclear leukocytes (PMNs), arachidonic acid metabolites (PGE2, etc), and other cellular components/products. Seltzer et al (1986) were the first to show acute lung inflammation results from ozone exposure using BAL technique. However the ozone concentrations chosen, 0.4 and 0.6ppm, are higher than typical U.S. concentrations. Devlin et al (1991) is one of the few studies investigating increase in inflammation markers at lower ambient ozone concentrations. In this study 10 healthy male volunteers were exposed to 0.10ppm and 18 volunteers to 0.08 ppm for 6.6 hours with intermittent 50 minutes of moderate exercise. BAL analysis was performed 16 hours after each exposure event. A significant increase in inflammatory markers was observed at both concentration levels with the greater increase corresponding to the higher ozone concentration. The duration of acute inflammatory response and time-course is not well understood (EPA 1996), but evidence suggests there is no attenuation of inflammatory damage resulting from repeat exposures to ambient levels of ozone (Jorres 2000). In other words, while respiratory responses like FEV1 and FVC may adapt
to repeat or prolonged ozone exposure, damage via inflammation may continue. This supports the theory that ozone induced acute airway inflammation can lead to (or indicate) permanent respiratory damage including altered immune response, increased sensitivity to environmental and biological pollutants, and/or development of respiratory disease (EPA 1996).

Results on the chronic health effects of ozone are less conclusive than that on acute effects. Most chronic health respiratory endpoints must be derived from a compilation of epidemiological and animal research. Epidemiology studies struggle to define true exposure-response relationships, especially at typical ambient ozone levels, but these studies remain the only means available to investigate chronic health endpoints in humans. Animal studies are limited by difficulties in extrapolating results into meaningful human health data. Nevertheless, the combination of both epidemiology and animal data suggests ozone exposure may cause a variety of chronic health endpoints including early degradation (accelerated aging) of lung tissue and increased incidence of airway disease.

Farman et al (1999) exposed 10-12 week old male Sprague-Dawley rats to 0.8ppm ozone, 14.4ppm NO₂, or both gases combined and compared lung morphometry to young rats exposed to filtered laboratory air. Each rat was exposed to gas for six hours per night for up to 90 days. All gas exposures resulted in centriacinar lung lesions, the most severe occurring in mixed gas exposure treatment. Sherwin et al (1991) investigated centriacinar lung damage in humans (ages 15-25). Data strongly suggested a relationship between ambient ozone levels and incidence of lung injury. Sherwin et al (2000) attempted to refine their study by comparing the lungs of youth and adults (ages 11 to 30, with an
available smoking history) from two cities, Los Angeles, CA (with many violations of the federal ozone standard) and Miami, Fl. (with few violations of the ozone standard). This study was limited by a small number of subjects, but results indicated higher centriacinar lesions in subjects from Los Angeles that could not be explained by smoking alone. Significant in both the two aforementioned studies is the young age of the subjects in which these lesions occur, suggesting chronic ozone exposure may lead to early degradation of lung tissue and potentially lung disease.

In light of Sherwin et al (1991) results, Costa et al (1995) et al investigated the effects of chronic ozone exposure on rats, in an attempt to elucidate the link between acute respiratory effects and the development of chronic lung disease. Seventy-two male Fischer rats (60-days old) were exposed to an urban (modeled after Los Angeles, CA) profile of ambient ozone for one of five exposure durations: 1, 3, 13, 15, or 72 weeks. Although link between acute effects and respiratory disease was not clear, data indicated ozone exposure leads to restrictive airway disease in rats, preceded by a variety of acute effects.

McDonnell et al (1998) investigated the link between chronic ambient ozone exposure and the incidence of asthma in non-smoking human adults over across 15 years (1977-1992) period. This prospective cohort study, including 3091 non-smoking adults (ages 27-87) in California, found a significant relationship (relative risk = 2.09) between doctor diagnosed asthma and average ambient 8-hour ozone concentrations. The study was weakened by lack of individual exposure data, but combined with Costa et al (1995) and similar studies, there is a strong indication that chronic ozone exposure leads to lung disease.
Calderon-Garciduenas et al (1992) demonstrated that ozone exposure might also lead to chronic health impacts in the nose. Nasal histology samples were compared between residents living in SW Mexico City (high ozone pollution) longer than two months, residents living in SW Mexico City less than 30 days, and residents living in Veracruz, Mexico (low ozone pollution) longer than five years. Abnormalities in nasal mucosa of the “longer” Mexico City residents were significantly greater than those of the “shorter” Mexico City residents, which in turn were significantly greater than abnormalities in nasal histology of Veracruz residents. The same nasal tissue degradation has not been observed in United States populations, likely because the US is not subject to the same levels of photochemical pollution.

Prior to 1997 the US EPA upheld a 1-hour ozone 0.12ppm standard. Due to extensive research demonstrating adverse respiratory impacts at 0.12ppm, The US EPA replaced the 0.12ppm with a 0.08ppm standard. This 8-hour standard means that the average annual ambient ozone concentration must not exceed 0.08ppm during an 8-hour time frame. This new standard went into effect January 2000. There is no research yet assessing population changes in respiratory symptoms or disease affected by the change in ozone standard. In addition there is continued concern about respiratory impacts of chronic low-level ozone exposures (US EPA 1996).

The Nose: Nasal Structure and Function

The nose is the beginning of the respiratory system serving two fundamental functions: (1) olfaction, and (2) modification of inspired air. The latter of these two is particularly important because the nasal mucosa is often the first respiratory area to come
into contact with outside air and contaminants. Modification of air, or "air conditioning",
requires changing the humidity and temperature as well as removing particles and other
environmental or biological pollutants from inspired air before it reaches the lungs.
Alveolar cells function best in highly humidified, close to 37°C, foreign particle and gas
free conditions (Cole 1987a). The complex geometry of the nose and the nasal mucosa
dictate airflow in such a way that the necessary physical and chemical processes needed
to “clean” the inspired air can occur, thus protecting the lower airways from atmospheric
contaminants.

The two main nasal passageways are slit-like irregular shaped tubes (Figures 1 and 2),
flat on one side and domed on the other (Olson et al 1987). They are separated by the
septal wall, and are approximately 10 centimeters in length (Mygind and Dahl 1997). The
nasal passageways are lined by epithelium, coated with a sticky mucus layer. The rigid
and bony anterior, middle, and inferior turbinates protrude into the nasal airways, both
altering airflow and resistance and increasing mucosal surface area (Cole 1987b).
At the anterior end of the nasal airways is an open “chamber” called the nasal vestibule.
The vestibule is coated with stiff hair useful for trapping large particles. Approximately
2 cm from the external opening, the nasal airway constricts down to a point called the
nasal valve (Corey et al 1997). The nasal valve is the narrowest cross-sectional area
within the nasal passageway. The nasal valve is the major “resistive segment” of the
nasal passageway (Cole 1987b) and dictates the flow condition of inspiratory air (Swift
1977). Research shows the nasal valve is responsible for near 50% of airway resistance
in the whole respiratory system (Mygind and Dahl 1997). Thus, change in the valve area
potentially has a major impact on the function of the entire respiratory system.
The nasal valve causes airflow velocity to significantly increase, causing inspired flow to change from laminar to intermediate at resting respiration. Laminar flow describes smooth "gentle" flow without mixing of airstreams. Intermediate flow is the transition from smooth flow to a more turbulent regime with air stream mixing and eddies. Beyond the nasal valve, the passageways widen into an area called the nasal cavum. The intermediate airflow allows the air stream to come in contact with nasal walls. Particles, water-soluble gases, and reactive gases typically become trapped or absorbed by the mucus lining. In addition, the close contact between the air stream and the mucus lining allows for heat and water exchange to occur (Cole 1987a). Jutting turbinates create further areas of "constriction", although none as small as the nasal valve, further influencing intermediate and possible turbulent airflow. Airflow transitions from intermediate back to laminar as it travels towards the pharynx, the termination of the nasal passages.

Nasal physiology changes during exercise. However, with higher nasal airflow associated with exercise ventilation, the flow regime is even more intermediate and turbulent than described above. Exercise naturally dilates the nasal airways (Portugal et al 1997) and reduces nasal resistance (Olson et al 1987). Nasal breathing during exercise has been observed in humans up to 60l/min flow rate, beyond which respiration switches from nasal to oronasal (Niinimaa et al 1980). It is unclear what causes the switch to occur, but once oronasal breathing begins, inspired air is no longer as efficiently filtered and modified before entering the lower respiratory tract.

There are a number of assessment techniques used to better understand nasal structure and geometry. Traditionally, techniques like rhinomanometry, computed tomography
(CT), or magnetic resonance imaging (MRI) have been used, but in the past decade
acoustic reflection has risen in popularity for clinical/research assessment of the nose.
Acoustic reflection was first applied to the respiratory system by Jackson et al (1977).
This research used sound wave pulses to estimate cross sectional areas of the proximal
airways of the lungs. Acoustic reflection has since been refined to measure the cross
sectional areas of the nose, hence the name acoustic rhinometry (AR). In this technique a
wave tube repeatedly emits a sound pulse of known wavelength into the nasal passage. A
microphone within the tube collects the incident and reflected sound waves. A computer,
then, converts the collected sound waves into an area-distance function. The result is a
rhinogram (Figure 3) a graphical representation of the nasal cross sectional area (y-axis)
by distance along the nasal passage (x-axis) (Hilberg 2002).

Hilberg et al (1989) were the first to describe acoustic rhinometry in detail. In this
study, acoustic rhinometry measurements on a nasal cast, a cadaver, and ten healthy
adults were compared with three other nasal geometry measurement techniques, CT,
anterior rhinomanometry, and a water measurement method. The authors found high
correlations (r>0.9) between AR and CT and rhinomanometry methods respectively, and
good agreement between AR and the water measurement technique. Overall, Hilberg et
al (1989) concluded that AR is a highly reproducible and accurate measurement of nasal
geometry. Since then, researchers have conducted many AR validation studies showing
both the high reproducibility of AR measurements as well as reasonable correlation with
other nasal assessment techniques (Hilberg 2002).

Recently Corey et al (1998) set normative standards of cross sectional areas (CSA) by
race for AR measurements. This study evaluated 160 subjects and compared the
minimum cross sectional area (MCA) and the second and third minimum cross-sectional areas (CSA2 and CSA3), each of which correspond to the nasal valve, the anterior edge of the nasal turbinates and the posterior edge of turbinates respectively (Lenders and Pirsig 1990) (Figure 3). They found a moderate difference between the cross-sectional values of males and females, as well as between Blacks, Caucasians, and Asians. These values have been incorporated into the EcoVision software (developed by Hood Laboratories) used in this study.

Endogenous nitric oxide (NO) is a highly reactive biologic molecule that has a variety of important cellular functions throughout the body (Jorissen et al 2001). The presence of NO in expired air was first discovered in 1991 (Gustafsson et al 1991). Since then there has been considerable research effort to understand where and why nitric oxide is produced in the respiratory system.

Measurements comparing upper and lower respiratory system show that the majority of exhaled nitric oxide is released from the nose (Lundberg et al 1994). Further research indicates nasal nitric oxide is primarily produced in the paranasal sinuses, although some nasal nitric oxide is produced in epithelial cells of the nasal passages (Lundberg et al 1997, Lewandowski et al 1998). The purpose of nasal and respiratory nitric oxide as whole, however, is not well understood. Preliminary research suggests respiratory nitric oxide may be important in host defense and inflammation (Jorres et al 2001), but the evidence is not conclusive.

The relative amount of released nitric oxide is influenced by a variety of physiologic (e.g. blood flow, ventilation, etc) and environmental (e.g. smoking) factors (Jorres et al 1999). Important in this study are the relationships between endogenous nasal nitric oxide
and (1) exercise and (2) reaction with ozone. Several studies have investigated endogenous nasal nitric oxide output and exercise. Lundberg et al (1994) observed a 47% decrease in nasal nitric oxide after one minute of heavy exercise (245 Watts) and 76% nasal nitric oxide reduction after five minutes of heavy exercise. Phillips et al (1996) calculated decreasing nasal nitric oxide release across seven regimes including rest condition (0W), light exercise (25W), moderate exercise (50 and 75 W), and heavy exercise (100 and 150 W). Although nasal nitric oxide output decreases with increasing exercise, it still comprises the majority of total respiratory nitric oxide (Lundberg et al 1994, Phillips et al 1996).

As discussed previously, nitric oxide readily reacts with ozone. Recently, Nightingale et al (1999) investigated the impact of ozone exposure on nasal nitric oxide. Exhaled oral and nasal nitric oxide production were compared between healthy subjects exposed to clean air or 0.2ppm ozone, respectively, for four hours. The study concluded that ozone exposure did not impact nasal nitric oxide production. There is no research, however, on whether endogenous nitric oxide influences uptake of ozone in the respiratory system.

Nasal Uptake of Ozone

Yokoyama and Frank (1972) were among the first to compare the oral and nasal uptake of ozone. Eleven dogs (mixed male and female) were exposed to two ozone concentration ranges (0.2-0.4 ppm and 0.7-0.8 ppm) across two flow ranges (3.5-6.5 L/min and 35-40L/min). The authors also compared ozone uptake in normal noses to uptake in decongested noses. One hundred and eighteen exposures were conducted in all. The dogs were anesthetized, and either their upper or lower airways were isolated and
mechanically ventilated. Results pertinent to nasal uptake of ozone included: (1) ozone uptake was higher in the nose than in the mouth for all ozone concentration ranges, (2) nasal uptake of ozone decreased with increasing flow, (3) nasal uptake of ozone decreased with increasing concentration, and (4) the changes in nasal uptake in the nose were random and did not exceed a greater than 10% between normal and decongested states.

Santiago et al (2001) investigated human nasal absorption of ozone. The researchers proposed two main hypotheses: (1) diffusion-reaction processes control ozone uptake in the nasal mucosa, and (2) during short exposure durations, ozone absorption is independent of ozone concentration. Ten healthy, non-smoking men and women underwent two exposure regimes altering flow and ozone concentration. The first measurements involved 0.4ppm ozone exposure at four flow rates: 3, 5, 8, and 15 l/min. For the second exposure regime flow was held constant at 15 l/min while subjects were exposed to 0.1, 0.2, and 0.4 ppm ozone. Subjects were required to raise their soft palate in order to isolate the nasal airways. The subjects then inserted two “pillows” into their nostrils and ozone was pulled through the right nostril and out of the left nostril. Ozone sampling ports were placed before entrance into the right nostril and after left nostril exit. The researchers used acoustic rhinometry to assess minimum cross sectional area and nasal volume. Results of the first exposure regime demonstrated that the fraction of nasal ozone uptake decreased from 0.8 to 0.33 with increasing flow rate. Second, there were small but significant decreases in fractional uptake with increasing ozone concentration. The researchers found no relationship between nasal volume, as measured by acoustic rhinometry, and fractional ozone uptake.
Three important areas to discuss in the Santiago et al (2001) study are (1) respiratory dynamics, (2) nasal physiology and (3) endogenous nitric oxide production. The authors assumed flow through one nostril would be approximately one half total respiratory flow during tidal breathing. However, dynamics of the nasal cycle suggest flow rate through both nostrils during natural respiration may not be equal, hence, the extent of ozone uptake may be impacted. Second, this study pulled air "forward" through one nostril, but "backward" through the second nostril to mimic inhalation and exhalation. It is known that nasal passages are not symmetrical, but the researchers found no significant difference in uptake based upon which nostril was used for "inhalation" versus "exhalation". However, they did not investigate impact of physiologic changes (such as those caused by exercise) on ozone uptake. Thirdly, the authors recognized endogenous production of nitric oxide might impact ozone uptake in nasal passageways. Nitric oxide measurements were not made in this study to determine the extent of impact of endogenous nasal nitric oxide on ozone uptake.

Purpose of Study

The nose protects the lower airways against inhaled particles and gases. Exercise naturally dilates the airways prior to the switch from nasal to oronasal respiration, as well as decreases endogenous nitric oxide concentration. Currently, there is no information on the impact of exercise-induced changes on nasal uptake of ozone. Thus, the purpose of this study was to examine the impact of moderate exercise on nasal absorption of ozone in healthy adults.
MATERIALS AND METHODS

Human Study Volunteers

Twenty-two men and women were recruited from in and around the area of Chapel Hill, North Carolina. Each subject signed an informed consent form approved by the UNC School of Medicine’s Committee on the Protection of Human Rights of Human Subjects, University of North Carolina at Chapel Hill. All potential subjects were required to be between the ages of 18 and 35 with 1) no history of smoking within the last five years and no greater than 0.2 pack-years prior to the past five, 2) no history of lung disease including asthma, fibrosis, or chronic obstruction, 3) no history of chronic coughing and/or wheezing, 4) no history of eardrum, middle ear, sinus, or head/neck surgery during the last five years, 5) no history of nasal surgery or obstructions, 6) no history of regular recreational drugs inhaled orally or nasally, 7) no current prolonged use or use within four weeks of nasal steroids, antihistamines, or decongestants, and 8) be free of upper and lower respiratory infections for four weeks prior to the study. Subjects meeting the aforementioned requirements, then underwent a screening which included; 1) a brief medical history, 2) training for the breath-holding maneuver required to make the ozone uptake measurements described below, 3) spirometry and body plethysmography pulmonary function tests, 4) acoustic rhinometry, 5) nasal nitric oxide production, and 6) a maximal exercise capacity test on a cycle ergometer. The maximal exercise test required fifteen minutes of exercise on a bicycle ergometer while pedaling at a rate between 60 and 70 revolutions per minute. The fifteen minutes was split into three graded and increasing five-minute sub-maximal exercise workloads (in watts) as heart rate was monitored by a 3 lead ECG. Heart rate at any of the three workloads did not exceed 170
beats per minute. A subject's maximum physical work capacity (PWCmax) was then predicted via linear extrapolation of the exercise workload (in watts) – heart rate relationship to individual predicted, age-related, maximum heart rate (Niinimaa et al 1980, Astrand 1977).

The breath-holding maneuver required subjects to hold their breath while closing the epiglottis (See Figure 4). Normally, this tends to be an involuntary response that causes upper airways to be isolated from the lower airways (e.g. prior to a cough in order to increase pressure in the thorax). In order for ozone uptake in upper airways to be measured, the epiglottis must be closed for a maximum of five seconds in order to get adequate uptake data. Twelve subjects could not perform the breath-holding maneuver required to make the ozone uptake measurements, so they were dismissed from the remainder of the study. Eight women and two men remained in the study; their anthropomorphic data are presented in Table 2.

Ozone Uptake Measurement

To measure nasal uptake of ozone, we used a nasal ozone exposure system (Figure 5) in which ozone laden air was pulled through a subject's nose and out through his/her mouth, while the subject kept their epiglottis closed. A 0.2ppm ozone concentration was generated by a photometric ozone calibrator (API Inc. San Diego, CA) and stored in a sixty liter Teflon bag. Teflon tubing led from the storage bag to a pneumotachograph (brand, type) and ultimately to two hollowed-out nose plugs that the subject inserted into each nostril. Subjects clamped their lips around a Teflon mouthpiece, which filled the mouth cavity. Tubing connected the mouthpiece with an air
vacuum pump and power supply, used to generate airflow. Flow rate was regulated by two rotometers placed between the air vacuum pump and the mouthpiece. The actual flow rate entering the nose was measured by the pneumotachograph connected to a pressure transducer (Validyne Engineer Corp.; Northridge, CA). The signal from the pressure transducer was amplified by a Validyne power supply. Ozone sampling ports, controlled by an electrical switch, were positioned so as to sample the ozone concentration entering the nostrils and leaving the mouth. The sampling ports were connected via Teflon tubing to a fast acting ozone analyzer to determine the ozone concentration in the sampled air. Gerrity et al (1995) described this ozone analysis system in detail. The sampled air is sent to a reaction chamber where it is mixed with ethylene. Any ozone in the air reacts with the ethylene and produces photons. A photomultiplier tube counts these emitted photons. The output is then converted into volts by a current amplifier. Both flow and ozone concentration signals (in volts) were acquired in real time on a MacLab data acquisition system for later analysis. Volts were converted to parts per million (ppm) and liters per minute (l/min), respectively, based upon calibration prior to each subjects visit. The percent nasal uptake of ozone was calculated as follows:

\[ \% \text{ Uptake} = (1 - [O_3] \text{ exiting the mouth/}[O_3] \text{ entering the nose}) \times 100\% \]

Acoustic Rhinometry

Acoustic Rhinometry (Hood Laboratories, Pembroke MA.) was used to assess nasal cavity geometry. A sound pulse, emitted from a wave tube, was sent into the nasal cavity, via a nose tip inserted a few millimeters inside a nostril. The nose tip was coated
with vaseline in order to ensure a good acoustic seal. The microphone within the wave
tube recaptured the incident and reflected sound waves from the nasal cavity. EcoVision
Software (Version) converted the collected wave signals into a rhinograph depicting
nasal cavity cross-sectional area (CSA) by distance. The EcoVision software also
calculated nasal volume and resistance values between selected points within the
rhinograph (Hilberg 1989). For any given AR assessment we performed two
measurements on each of a subject’s right and left nostrils. Variability between these
measurements was less than 7%. MCA, CSA2, nasal volume, and nasal resistance values
from each of the four measurements were averaged.

Nitric Oxide Measurement

Nasal nitric oxide (NO) concentration was measured with Nitric Oxide
Chemiluminescence Analyzer, Model 270B (Siever Corp, Boulder Co.) A sample line is
connected from the nitric oxide analyzer to one nostril via a nasal plug, while the other
nostril is open to ambient air. Maximum nitric oxide production is determined when the
subjects close off their soft palate (Figure 4), by making a “k” sound. This separates the
nasal passages from the rest of the respiratory system, and helps ensure observed nitric
oxide concentration is purely from the nasal passage. The sampled nitric oxide is
recorded as a voltage signal from the nitric oxide analyzer that is calibrated prior to every
subject’s study day, i.e., the sample data is converted by MacLab data acquisition system
to micrograms of nitric oxide per liter-minute. nitric oxide concentration is sampled from
both the right and left nostrils of each subject.
Experimental Protocol

Fractional ozone uptake measurements before and after exercise were conducted on all the subjects that met the requirements for this study. Prior to ozone uptake, baseline measurements, acoustic rhinometry (AR) and nasal nitric oxide (NO) measurements were completed. Each subject then underwent the first nasal ozone uptake measurements exposure using the ozone exposure/measurement system described above. Ozone uptake measurements were repeated three times at each of two flow rates, 10 l/min and 20 l/min. Next, each subject spent fifteen minutes exercising, between 40 and 60 percent of their PWCmax (determined during screening) on a bicycle ergometer. AR and nitric oxide measurements were conducted immediately after bicycle exercise to assess any changes in these nasal characteristics. Nasal uptake measurements were then repeated, again three measurements at both 10 and 20 liters per minute. The time between each measurement was recorded to ensure constancy between subjects. Immediately following the ozone exposure/measurements, AR and NO measurements were repeated to ensure ozone exposure or the time required to make the measurements did not cause changes in nasal geometry or nitric oxide concentration. The sequence of the measurement events is detailed in Table 1.

Data Analysis

All data were compiled in Excel for preliminary analysis. The statistical package SPSS was used to conduct paired T-tests to 1) analyze ozone uptake, nitric oxide, and acoustic rhinometry values before and after exercise, and (2) verify that nitric oxide and acoustic rhinometry values were not affected by ozone uptake measurements. Regression analysis
was conducted to examine acoustic rhinometry parameters and nitric oxide production as predictors of ozone uptake. Analysis of nasal volume, acoustic rhinometry parameters and nitric oxide were conducted pre and post ozone exposure (both pre and post exercise: See Table 1) in order to evaluate exposure related or temporal biases.
RESULTS

The anthropomorphic data on the ten subjects included in this study are reported in Table 2.

Ozone Uptake

Inter-subject variation in mean ozone uptake ranged from 26.8% to 64.8% pre-exercise. Post-exercise the inter-subject variation ranged between 27.2% and 65.4%. The results of the paired t-tests for mean nasal absorption of ozone are recorded in Table 3 and Figure 8. There was a significant difference ($p = 0.00, \alpha = 0.05$) in ozone uptake between the two flow rates, 10 l/min and 20 l/min both pre- and post-exercise. Ozone uptake at 10 l/min in both pre and post exercise measurements was nearly 50% greater than that at the 20l/min flow rate.

There was a slight, but statistically insignificant increase in mean uptake at 10l/min post exercise when compared to pre-exercise. At 20 l/min pre- and post exercise, there was not a significant difference in the mean percent uptake of ozone in the nose.

To determine the approximate amount of ozone delivered to the lung, a dose rate was calculated as follows:

$$\text{Dose Rate (to lung)} = \text{Flow Rate} \times [\text{O}_3] \times (100-\% \text{ Nasal O}_3 \text{ Uptake})$$

Pre-exercise the dose rate to the lungs was 0.89 ppm·l/min and 2.53 ppm·l/min at 10 and 20 l/min respectively. Post-exercise the dose rate at 20 l/min was 0.91 ppm·l/min, and 2.53 ppm·l/min at the 10l/min flow rate. Both pre- and post-exercise, the dose rate at the high flow rate (20 l/min) was approximately 1.6 times higher than the dose rate at the low flow rate (10 l/min).
Acoustic Rhinometry and NO Measurements

Five acoustic rhinometry values and nasal nitric oxide production, listed in Table 4, were compared between pre- and post- exercise, as well as the percent changes in these parameters. Nasal volume (Vn) and nasal resistance (Rn) were determined for the area extending between the first minimum cross-sectional area and the third minimum cross-sectional area. There was significant increase in volume and all three evaluated minimum cross-sectional areas post-exercise. Nasal resistance significantly decreased post-exercise by 63.7 percent. There was a 14% decrease in nasal nitric oxide post-exercise, but this finding was not statistically significant (p = 0.10).

Regression Analysis

Four single predictors of nasal uptake of ozone, nasal volume (Vn), resistance (Rn), NO, and CSA2: MCA ratio, were analyzed using simple linear regression. Analysis of each rhinograph demonstrated that the CSA2: MCA ratio might best represent the rapid changes in flow velocity and associated flow separation that may occur downstream of the nasal valve. There was no significant correlation between any of the pre- or post-exercise predictors and nasal uptake of ozone. In other words, these values were not useful for predicting intersubject variability in ozone uptake.

On the other hand, the percent change in nasal ozone uptake (from pre-exercise to post-exercise) regressed on each of the percent changes in Vn, Rn, CSA2:MCA, and NO showed some statistically significant correlations (Tables 5 and 6). These statistically significant linear relationships are graphically represented in Figures 8 and 9. At 10 l/min
there was a significant linear relationship between percent change in ozone uptake post-exercise and both the percent changes in volume and CSA2: MCA post-exercise. At 20 l/min, there was also a significant linear relationship between percent change nasal uptake and percent change in nasal volume, but not with percent change in CSA2: MCA.
DISCUSSION

The aim of this study was to determine the effect of exercise induced flow and physiologic changes on the absorption of ozone in the nose. In agreement with a previous study, Santiago et al (2001), there was a significant effect of flow rate on nasal absorption of ozone. Mean nasal absorption of ozone at 20 l/min was significantly less (approximately 34% less both pre- and post-exercise) than the mean ozone absorption at 10l/min. However, our mean fractional ozone uptake at 10 l/min (55%) and 20l/min (36%) were slightly lower than values Santiago et al (2001) recorded at similar flow rates. This discrepancy is likely caused by differences in measurement technique. Santiago et al (2001) pulled ozonated air through one nostril and out through the second nostril. Because nasal flow characteristics are different upon expiration than inspiration, the rection between ozone and the mucus layer may also be different. This may explain the slightly higher values of ozone uptake in the Santiago study. Santiago et al (2001) 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. The nasal cycle is the change in nasal resistance, shifting between left and right nostrils, over time. Therefore, doubling selected flow rates (for comparison of our data to theirs the Santiago et al (200) data) may be an inaccurate estimate of true inspiratory flow-rate during normal breathing conditions. The consequence of this is that it remains uncertain what exact inspiratory flow rate corresponds to the measured fractional absorption significant of ozone in their study. Our
study addresses this problem by comparing concentrations before entering both nostrils with the concentration exiting the mouth.

Also like Santiago et al (2001), this study found no significant inter-subject relationship between nasal volume and nasal absorption of ozone at either 10 or 20 l/min. In theory, ozone uptake should be proportional to the transit time through a defined airspace. Increasing the nasal volume, then, should increase the transit time and ultimately increase ozone uptake. There are several plausible reasons why this relationship was not observed. First, as evident from Swift and Proctor (1977) and Cole (1982), airflow through the nasal cavity may not encompass the entire volume of the nasal cavity (Figures 2 and 6). As a result, measurements on the total increase in nasal volume may not truly reflect the relative increase in volume along the main airflow passageways. Total nasal volume, therefore, may be an insensitive parameter by which to predict percent ozone uptake. In addition, this study did not examine all effects of exercise on nasal physiology. Just as nitric oxide is produced in the nose and is affected by exercise, so are other biochemical components of airway secretion that may react with ozone. As discussed by Santiago et al (2001) the airways produce antioxidant species such as uric acid and glutathione that readily react with ozone. Exercise induced changes in the secretions of these components may counteract any effect that increased nasal volume may have on nasal ozone absorption. A third explanation deals with the relatively small changes in nasal volume we observed with exercise. The average percent change (increase) in volume across all subjects was only 30%. With greater nasal dilation (i.e., greater changes in percent volume) there may be an observable volume effect on ozone uptake. This possibility was explored by plotting the percent change in volume
versus the percent change in nasal ozone uptake at each flow rate. Strong correlations between percent change in volume and percent change in ozone uptake were observed at both levels. It is important to note, however that the impact is very small. The highest percent change in volume, 71 percent, affected no greater than 20% and 6% increase in nasal ozone uptake at the 20l/min and 10l/min flow rates respectively. In addition, this 71% change in volume was an outlier. The relationship between percent change in volume and percent change in uptake loses statistical significance without this value. Also important in this analysis is that percent change in uptake is negative at lower percent changes in volume for both flow rates. This suggests that there are other factors tending to reduce nasal ozone uptake following exercise. For example, reduced nitric oxide production following exercise may tend to reduce ozone uptake. We did find a tendency for nitric oxide to be decreased following exercise, but the reduction was not statistically significant. Other studies (Lundberg et al 1994, Phillips et al 1996), with an expanded study base however have found statistically significant reductions in nitric oxide following exercise. However, we found no correlation between the percent change in uptake versus percent change in nitric oxide as we observed versus the percent change in volume and CSA2: MCA (Figures 8 and 9).

This study went further than previous studies by also investigating the relationship between exercise-induced changes in nasal resistance, MCA, CSA2, and nitric oxide production on nasal absorption of ozone. Pairwise comparisons of these values before and after exercise demonstrated significant changes among all parameters except for endogenous nitric oxide production, though, post-exercise nitric oxide tended to be lower than pre-exercise nitric oxide values. In spite of this, there was no significant
relationship between nasal absorption of ozone at either the 10l/min or 20l/min flow rates and any of these chosen parameters. A statistically significant relationship emerged when comparing the percent change in ozone uptake with the percent changes in CSA2/MCA (Table 5 and 6). Like the percent change in volume analysis, the percent change in CSA2/MCA affected minimal percent change in ozone uptake (<20%). (See Figure 9) The use of two or more predictors, simultaneously in the regression analysis may provide more insight into this situation. However the small sample size (n=10) in this study, made the use of multiple predictors impractical.

To make certain that the ozone exposure did not affect the acoustic rhinometric and nasal volume endpoints, pairwise comparisons were made between the pre-exercise, pre-ozone exposure parameters (See Tables 1 and 7). There were no statistically significant differences between any of the chosen study parameters pre- and post- ozone exposure. To determine whether nasal dilation associated with exercise (represented by nasal volume and AR parameters) diminished over time (i.e. the time that passed between the first and second post-exercise measurements) comparisons were made between the post-exercise, pre-ozone data and the post-exercise, post-ozone data (See Table 8). Mean nasal volume post-exercise, pre-ozone was significantly greater than nasal volume post-exercise, post-ozone. This indicates that total nasal volume was decreasing during the post-exercise ozone exposure maneuver. Comparison between the final nasal volume measurement (post-exercise, post-ozone) and the baseline nasal volume measurement (pre exercise, pre-ozone), demonstrates that the final nasal volume was not significantly greater (p = 0.30) than the baseline nasal volume. This may bias nasal uptake of ozone differences between pre- and post exercise towards the null.
The measurement technique, breath holding by closing the epiglottis, was too
difficult for 50% of the screened subjects. It is possible the dismissed subjects simply
were not able to sustain a closed epiglottis for longer than a few seconds. Another
explanation is that while subjects were closing the epiglottis they were also closing their
soft palate. The latter closes off the nasal passageway from the oral cavity and thus does
not allow airflow into the nose and out of the mouth. To determine whether the
measurement technique requirements caused selection bias, an analysis of nasal
resistances between selected subjects and dismissed subjects was conducted. No
significant difference was observed.

A few research studies have compared ozone absorption efficiency of the nasal
and oral cavities (Gerrity et al 1988) and consequent health effects (Hynes et al 1988,
healthy adult males. Eighteen young men between the ages of 18 and 35 were exposed to
three ozone concentrations, 0.1, 0.2, and 0.4 ppm within an environmental chamber.
Subjects were instructed to breath in one of three manners: nose only, mouth only, or
oronasally at 12 or 24 breaths per minute (approximately 21 and 38 l/min respectively). A
pediatric feeding tube ran through one nostril into the posterior pharynx in order to
sample airway ozone concentration. Unlike Santiago et al (2001), the Gerrity et al
(1988) study found no concentration dependence in ozone uptake efficiency, though
uptake tended to decrease with increasing concentration. On the other hand, Gerrity et al
(1988) did observe a significant difference (p < 0.001) in uptake at the two different
breathing rates, in agreement with both the Santiago study and our current study.
However, uptake at 24 bpm was only 6% less than at 12 bpm, a difference much smaller than the difference in uptake observed in the Santiago et al (2001) and in our study.

As for nasal versus oral inhalation of ozone, Gerrity et al (1988) found no significant difference between ozone uptake via nasal (40.4%) and oral (44.5%) exposure routes. This contradicts the findings of the Yokoyama and Frank (1972) study that reported the ozone removal efficiency in dog noses as nearly 50% more efficient than in the mouth. If Gerrity et al (1988) results in humans are true then they would invalidate the assumption that the nose protects the lower airways from ozone exposure. However, the ozone measurement technique used in the Gerrity et al (1988) study likely biased their results away from the null. The insertion of the pediatric tube through the nostril may block natural airflow. Therefore, nasal and oronasal respiration may have occurred primarily through the use of one nostril. This would tend to reduce ozone absorption during nasal and oronasal respiration (i.e. equivalent to increasing flow rate through the nose). Secondly, Gerrity et al (1988) did not investigate whether or not ozone measurements were affected by the position of the sampling tube relative to the inspiratory and expiratory air stream within the pharynx. If the tip of the sampling tube was not centered within a well-mixed air stream, collected data may represent localized ozone concentrations near the airway wall. The position of the sampling tube in the nose and in the pharynx may have confounded the conclusions in the Gerrity et al (1988) study. Lastly, the chosen flow rates, particularly 38 l/min, are fairly high. The nose absorbs ozone less efficiently at higher flow rates (Santiago et. al. 2001). Gerrity et al (1988) may have not observed difference in ozone between the mouth and nose because chosen flow rates are in a range where nasal uptake reaches a minimum plateau.
Adams et al (1989) and Hynes et al (1988) both investigated FEV response to 0.4ppm ozone, comparing the difference in FEV response from oral and nasal exposure. Hynes et al (1988) exposed healthy young adults for 30 minutes during moderate continuous exercise protocol. Adams et al (1989) exposed healthy young males during moderate and heavy continuous exercise. In these two studies, the researchers found no significant difference in FEV response to 0.4ppm ozone exposure based upon route of exposure. Results from Adams et al (1989) and Hynes et al (1988) support the hypothesis that there is little difference in ozone uptake efficiency between the oral and nasal respiration routes. There are, however, two important discussion points that apply to both the Adams and Hynes studies: (1) chosen ozone concentration, and (2) chosen health effect endpoint. Their selected ozone concentration is higher than most U.S. ambient ozone concentrations, but was likely chosen because 0.4ppm is known to cause decreases in forced expiratory endpoints. Yet, this higher concentration may be near the threshold FEV response. As a result, researchers may not have been able to distinguish the FEV responses between the two exposure routes. To verify results in these studies, health impacts categorized by exposure route should be investigated over a range of ozone concentrations, for example, from ozone-free air to 0.4ppm. Secondly, FEV changes are an acute response to ozone exposure. The oral versus nasal breathing route of exposure may be more important in chronic health effects. Thus, future study should focus on airway inflammation or other respiratory responses associated with chronic health endpoints rather than FEV alone.

Another area for future study suggested from our results is gender differences in nasal ozone uptake. This current study included eight females and two males. Both males
exhibited the highest percent change (post-exercise) in nasal volume (71.5% and 52.2%) as well as the highest percent change (post-exercise) in acoustic rhinometry ratio (41.5% and 32.9%). These percent changes, when plotted against percent change in ozone uptake, standout from the remainder of the data (See Figures 8 and 9). Three potential research questions arise from this: (1) Is there a gender difference in baseline and exercise induced change in nasal ozone uptake; (2) If there is a gender difference, is it caused by differences in nasal physiology; and (3) If there is a gender difference, does it result in differential health risk from ozone exposure?

The third question has been addressed in part by Laupertzen and Adams (1985) and Seal et al (1993). The first study exposed six continuously exercising females to three ozone concentrations: 0.2, 0.3, and 0.4ppm. The decreases in forced vital capacity (FVC) and FEV1 in these women were dose-dependent and significantly greater than FVC and FEV1 decreases in men from a previous study (Adams et al 1981). However, researchers only looked at acute effects resulting from ozone exposure via oral inhalation. A similar, but not statistically significant response was found in the Seal et al (1993) study among Caucasian women and men, but only at 0.4ppm ozone. However, African-American men demonstrated elevated FEV1 responses to ozone exposure across five ozone concentrations, 0.12, 0.18, 0.24, 0.30, and 0.4ppm, in comparison to African-American women, Caucasian women, and Caucasian men. This latter finding suggests that not only gender, but also race may influence acute response to ozone. Like, Lauritzen and Adams (1985), however, Seal et al (1993) did not investigate markers of chronic health effects.
Conclusion

This study addressed two important issues in respiratory physiology and air pollution: (1) the impact of moderate exercise impact on the uptake of ozone in the nose; and (2) potential physiologic predictors of nasal ozone uptake. In agreement with previous studies, we found that nasal uptake of ozone, as a percentage of incoming concentration, increases with decreasing flow rate. Paired T-test analysis demonstrated that on average moderate exercise does not have an effect on nasal ozone absorption. This may be caused by unmeasured exercise-induced physiologic or biochemical changes that counteract the impact of the parameters measured in this study. On the other hand, significant flow rate effects were observed in this study. Our data demonstrated that the percent nasal uptake of ozone decreases with increasing flow. Flow rate increases with increasing exercise; therefore, the net effect would still be that even greater amounts of ozone, than otherwise expected, are introduced to the lower respiratory tract due to increased flow during exercise. Because ozone uptake is decreased at higher flow, the dose delivered to the lungs is increased in greater proportion. This data may be useful in human health risk assessment analysis for ozone exposure, employing various exercise ventilation flow rates, oral-nasal distribution of airflow, and estimates of nasal uptake to predict ozone dose delivered to the lung.
REFERENCES


Folinsbee LJ, DH Horstman, HR Kehrl, S Harder, S Abdul-Salaam, and PJ Ives, Respiratory responses to repeated prolonged exposure to 0.12 Ozone, American Journal of Respiratory and Critical Care Medicine, 149, pp 98-105, 1994


Gilner JA, SM Horvath, and LJ Folinsbee, Pre-exposure to low ozone concentration does not diminish the pulmonary function response on exposure to higher ozone concentrations, American Review of Respiratory Disease, 127, pp 51-55, 1983
Gong H Jr, MS Simmons, WS Lin, WF McDonnel, D Westerdahl, Relationship between acute ozone responsiveness and chronic loss of lung function in residents of a high-ozone community, *Archives of Environmental Health*, 53(5), pp 313-319, 1998


Hilberg O, Objective measurement of nasal airway dimensions using acoustic rhinometry: Methodological and clinical aspects, *Allergy* (Supplement 70), 57, pp 5-39, 2002


Horstman DH, LJ Folinsbee, PJ Ives, S Abdul Salaam, and WF McDonnell, Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. *American Review of Respiratory Disease* 142, pp 1158-1163, 1990


Laurtizen SK and WC Adams, Ozone inhalation effects consequent to continuous exercise in females: comparison to males, *Journal of Applied Physiology*. 59 (5), pp 1601-1606, 1985


Seltzer J, BG Bigby, M Stulbarg, MJ Holtzmann, JA Nadel, IF Ueki, GD Leikauf, EJ Goetzl, and HA Boushey, Ozone-induced change in bronchial reactivity to methacholine


U.S. Environmental Protection Agency: National Center for Environmental Assessment, Air Quality Criteria for Ozone and Related Photochemical Oxidants, EPA/600/P-93/004a-cF, July 1996

World Health Organization, Ozone and Other Photochemical Oxidants in Air Quality Guidelines, Regional Office for Europe Report, 2nd Edition, Copenhagen, Denmark, 2000

TABLES

Table 1 Visit schedule for participants in the study on effects of exercise on nasal absorption of ozone

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Procedure</th>
<th>NO and AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5</td>
<td>Pre Exercise, Pre Ozone Measurements (NO and AR)</td>
<td>A_a</td>
</tr>
<tr>
<td>6-16</td>
<td>Pre-Exercise Ozone Exposure and Uptake</td>
<td></td>
</tr>
<tr>
<td>17-22</td>
<td>Pre Exercise, Post Ozone Measurements (NO and AR)</td>
<td>B</td>
</tr>
<tr>
<td>23-38</td>
<td>Bicycle Exercise</td>
<td></td>
</tr>
<tr>
<td>39-41</td>
<td>Post Exercise, Pre Ozone Measurements (NO and AR)</td>
<td>C</td>
</tr>
<tr>
<td>41-46</td>
<td>Post Exercise Ozone Exposure/Uptake</td>
<td></td>
</tr>
<tr>
<td>47-50</td>
<td>Post Exercise, Post ozone Measurements (NO and AR)</td>
<td>D</td>
</tr>
</tbody>
</table>

a. Measurements A and C were used for analysis of pre vs. post exercise changes in ozone uptake, while B and D were used to determine if ozone exposure or time lapse between measurements affected acoustic rhinometry and nitric oxide results.

Table 2 Anthropomorphic data on subjects retained for the study.

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>21</td>
<td>160</td>
<td>61.0</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>22</td>
<td>158</td>
<td>59.0</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>23</td>
<td>165</td>
<td>53.5</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>23</td>
<td>163</td>
<td>64.0</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>23</td>
<td>158</td>
<td>63.2</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>19</td>
<td>163</td>
<td>57.6</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>23</td>
<td>164</td>
<td>58.0</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>23</td>
<td>156</td>
<td>51.8</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>25</td>
<td>179</td>
<td>76.4</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>26</td>
<td>183</td>
<td>87.3</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>23 ± 2</td>
<td>165 ± 9</td>
<td>63.2 ± 10.9</td>
<td></td>
</tr>
</tbody>
</table>
### Table 3 Comparison of nasal uptake of ozone pre- and post-exercise, at two airflow rates, 10 and 20 l/min

<table>
<thead>
<tr>
<th>Pairs&lt;sub&gt;a&lt;/sub&gt;</th>
<th>% Uptake&lt;sub&gt;b&lt;/sub&gt;</th>
<th>Std. Dev</th>
<th>Correlation (r)</th>
<th>95% CI</th>
<th>T-value</th>
<th>Significance (2-tail) α = .05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre 10, Pre 20</td>
<td>55.7, 36.7</td>
<td>7.83, 4.93</td>
<td>0.88</td>
<td>(16.0, 22.0)</td>
<td>14.2</td>
<td>.001</td>
</tr>
<tr>
<td>Pos 10, Post 20</td>
<td>54.6, 36.8</td>
<td>7.97, 5.49</td>
<td>0.89</td>
<td>(14.9, 20.7)</td>
<td>14.1</td>
<td>.001</td>
</tr>
<tr>
<td>Pre 10, Post 10</td>
<td>55.7, 54.6</td>
<td>7.83, 7.97</td>
<td>0.96</td>
<td>(.057, 2.69)</td>
<td>1.47</td>
<td>.175</td>
</tr>
<tr>
<td>Pre 20, Post 20</td>
<td>36.7, 36.8</td>
<td>4.93, 5.49</td>
<td>0.88</td>
<td>(1.95, 1.75)</td>
<td>-0.12</td>
<td>.906</td>
</tr>
</tbody>
</table>

a. Paired t-tests were used to compare pre- and post-exercise values within the same subject

b. % Nasal Uptake = [(1 - [O<sub>3</sub> exiting the mouth)/ [O<sub>3</sub>] entering the nose] x 100%

### Table 4 Comparison of acoustic rhinometry and nitric oxide values pre- and post exercise

<table>
<thead>
<tr>
<th>Pairs&lt;sub&gt;a&lt;/sub&gt;</th>
<th>% Mean Uptake</th>
<th>Std. Dev</th>
<th>Corr. (r)</th>
<th>95% CI</th>
<th>T-value</th>
<th>Significance (2-tail) α = .05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Vol, Post Vol</td>
<td>7.65, 9.86</td>
<td>0.72, 1.06</td>
<td>-0.11</td>
<td>(-3.17, -1.24)</td>
<td>-5.18</td>
<td>.001</td>
</tr>
<tr>
<td>Pre R, Post R</td>
<td>0.980, 0.624</td>
<td>0.40, 0.24</td>
<td>0.79</td>
<td>(0.17, 0.54)</td>
<td>4.28</td>
<td>.002</td>
</tr>
<tr>
<td>Pre MCA, Post MCA</td>
<td>0.47, 0.55</td>
<td>0.10, 0.11</td>
<td>0.82</td>
<td>(-0.13, -0.04)</td>
<td>-4.30</td>
<td>.002</td>
</tr>
<tr>
<td>Pre CSA2, Post CSA2</td>
<td>0.92, 1.20</td>
<td>0.11, 0.19</td>
<td>0.50</td>
<td>(-0.40, -0.16)</td>
<td>-5.27</td>
<td>.001</td>
</tr>
<tr>
<td>Pre CSA3, Post CSA3</td>
<td>1.28, 1.74</td>
<td>0.16, 0.24</td>
<td>0.59</td>
<td>(-0.60, -0.32)</td>
<td>-7.45</td>
<td>.001</td>
</tr>
<tr>
<td>Pre NO, Post NO</td>
<td>333, 291</td>
<td>167, 129</td>
<td>0.92</td>
<td>(-9.08, 92.3)</td>
<td>1.86</td>
<td>.096</td>
</tr>
</tbody>
</table>

a. Units: Vol = cm<sup>3</sup>, Resistance = cmH<sub>2</sub>O/L/min, MCA = cm<sup>2</sup>, CSA2 = cm<sup>2</sup>, NO = nl/L/min
Table 5 Regression of percent change in acoustic rhinometry and nitric oxide values post-exercise on the percent change post-exercise in nasal ozone uptake at 10 l/min.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>r-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume*</td>
<td>0.70</td>
<td>0.02&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>Rn*</td>
<td>-0.17</td>
<td>0.63</td>
</tr>
<tr>
<td>CSA2/MCA*</td>
<td>0.82</td>
<td>&lt;0.01&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>NO</td>
<td>-0.26</td>
<td>0.47</td>
</tr>
</tbody>
</table>

a. Statistically significant at α= .05
b. Statistically significant at α= .01

Table 6 Regression of percent change in acoustic rhinometry and nitric oxide values post-exercise on the percent change post-exercise in nasal ozone uptake at 20 l/min.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>r-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>0.69</td>
<td>0.03&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>Rn</td>
<td>-0.52</td>
<td>0.32</td>
</tr>
<tr>
<td>CSA2/MCA</td>
<td>0.59</td>
<td>0.07</td>
</tr>
<tr>
<td>NO</td>
<td>0.13</td>
<td>0.71</td>
</tr>
</tbody>
</table>

a. Statistically significant at α= .05
Table 7 Bias and Confounding Analysis: Pre-Exercise Paired t-test Results for Acoustic Rhinometry and Nitric Oxide Measurements

<table>
<thead>
<tr>
<th>Pairs_{a,b}</th>
<th>Mean</th>
<th>Std. Dev</th>
<th>95% CI</th>
<th>T-value</th>
<th>Significance (2-tail) ( \alpha = .05 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol A, Vol B</td>
<td>7.65,</td>
<td>0.72,</td>
<td>(-0.51,1.66)</td>
<td>1.19</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>7.08</td>
<td>1.13</td>
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</tr>
<tr>
<td>Resist A, Resist B</td>
<td>0.98,</td>
<td>0.40,</td>
<td>(-0.43,0.23)</td>
<td>-0.67</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>1.08</td>
<td>0.51</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MCA A, MCA B</td>
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<td>0.10,</td>
<td>(-0.03,0.06)</td>
<td>0.78</td>
<td>0.45</td>
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<td></td>
<td>0.45</td>
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<tr>
<td>CSA2 A, CSA2 B</td>
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<td>0.11,</td>
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<td>0.84</td>
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<tr>
<td>NO A, NO B</td>
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<td>167,</td>
<td>(-3.12,32.1)</td>
<td>1.86</td>
<td>.096</td>
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<tr>
<td></td>
<td>319</td>
<td>148</td>
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</tr>
</tbody>
</table>

a. Units: Vol = cm^3, Resistance = cmH_2O/L/min, MCA = cm^2, CSA2 = cm^2, NO = \( \eta \)L/min  
b. Pair Lettering is According to Table 1.

Table 8 Bias and Confounding Analysis: Post-Exercise Paired t-test Results for Acoustic Rhinometry and Nitric Oxide Measurements

<table>
<thead>
<tr>
<th>Pairs_{a,b}</th>
<th>Mean</th>
<th>Std. Dev</th>
<th>95% CI</th>
<th>T-value</th>
<th>Significance (2-tail) ( \alpha = .05 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol C, Vol D</td>
<td>9.86,</td>
<td>1.06,</td>
<td>(0.12,3.21)</td>
<td>2.44</td>
<td>.04_c</td>
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<tr>
<td></td>
<td>8.19</td>
<td>1.54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resist C, Resist D</td>
<td>0.62,</td>
<td>0.24,</td>
<td>(-0.40,0.02)</td>
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<tr>
<td></td>
<td>0.81</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCA C, MCA D</td>
<td>0.55,</td>
<td>0.11,</td>
<td>(-0.01,0.12)</td>
<td>1.10</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>0.51</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSA2C, CSA2D</td>
<td>1.20,</td>
<td>0.19,</td>
<td>(-0.71,0.03)</td>
<td>-2.06</td>
<td>0.07</td>
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<tr>
<td></td>
<td>1.54</td>
<td>0.46</td>
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</table>

a. Units: Vol = cm^3, Resistance =cmH_2O/L/min, MCA = cm^2, CSA2 = cm^2, NO = \( \eta \)L/min  
b. Pair Lettering is According to Table 1  
c. Statistically Significant at \( \alpha = .05 \)
FIGURES

Figure 1. Lateral view of the nasals cavity (Mygind and Dahl 1997)

Figure 2. Nasal airway in mid-passage (Cole 1987)

This cross-section demonstrates the slit-like nature of the nasal passageways. Stippled areas indicate olfactory airway. The clear areas represent the main nasal airway, and the hatched areas the nasal meatuses.
**Figure 3. Rhinogram**

This graph is representative of the distance by cross-sectional area within the nasal cavity output of the acoustic rhinometer. Cross-sectional area "valleys" correspond to anatomical points within the nose.

**Figure 4. Nasal Cavity, Soft Palate, and Epiglottis**

**Medial Wall of Nasal Cavity (Nasal Septum)**

Lateral view of the nasal and oral passageways with the soft palate that closes of the nose from the remainder of the airways, and the epiglottis that closes of the oral and nasal passageway from the lower airways.
The vacuum pump pulls 0.2ppm stored O₃ at one of two flow rates (10 and 20 l/min) through a subjects nose and out of their mouth while they hold their epiglottis closed. Ozone concentration is sampled immediately before the nose and immediately after the mouth and stored on the MacLab data acquisition system for later analysis.
Figure 6. Linear Velocity of Inspiratory Nasal Airflow (Swift and Proctor 1977)

Lateral view of the nasal passageway. The relative increase in velocity of inspiratory airflow corresponds with increase in circle size. This demonstrates that bulk airflow may not reach all areas of the nasal cavity.
Figure 7. Nasal Uptake of Ozone versus Flow Rate
Figure 8 Regression of Percent Change in Ozone Uptake on Percent Change in Nasal Volume

$R = 0.69$

$R = 0.70$
Figure 9 Regression of Percent Change in Ozone Uptake on Percent Change in CSA2: MCA
APPLICATION FOR APPROVAL OF RESEARCH INVOLVING HUMAN SUBJECTS

DATE: 9 Nov 2001 IRB STUDY NUMBER (leave blank if new submission): ____

TITLE OF STUDY: The relationship between internal nasal structure and nasal uptake of ozone in healthy adults before and after exercise

NAME AND DEGREE(S) OF PRINCIPAL INVESTIGATOR: William D. Bennett, PhD DEPT: CEMLB
SOCIAL SECURITY NUMBER OF PRINCIPAL INVESTIGATOR: 523-82-0064
MAILING ADDRESS: CB 7310 104 Mason Farm Rd UNC-Chapel Hill 27599

PHONE: 966-6229 FAX: 966-9863 PAGER:
E-MAIL: William_Bennett@med.unc.edu

NAMES AND DEGREE(S) OF CO-INVESTIGATORS: Keegan Musgrove-Wesley BS, Milan Hazucha PhD, Todd Alter MS, and Philip Bromberg MD

NAME AND PHONE NUMBER OF RESEARCH COORDINATOR, IF APPLICABLE:

NAME OF FUNDING SOURCE: USEPA

I. Agreements

Principal Investigator:
I certify that each of the above-named co-investigators has accepted his/her role in this study. I agree to a continuing exchange of information with the Committee on the Protection of the Rights of Human Subjects (IRB). I agree to obtain IRB approval before making any changes or additions to the project. I will provide progress reports at least annually, or as requested. I agree to report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. A copy of the consent form will be given to each subject and the signed original will be retained in my files. If the study involves treatment of UNC Hospitals patients, a copy of the consent form will be placed in each subject’s medical record.

_________________________________________  __________________________
Signature of Principal Investigator             Date

_________________________________________  __________________________
Signature of Faculty Advisor if P.I. is student  Date

Department Chair of P.I. (or Vice-Chair if Chair is investigator or otherwise unable to review):
I have reviewed this research study. I believe the research is sound, that the study design and methods are adequate to achieve the study goals, and that there are appropriate resources (financial and otherwise) available to the investigator. I support it, and hereby submit it for further review.

_________________________________________  __________________________
Signature of Department Chair                  Department             Date

(Rev. 12/09/99)
### II. Summary Checklist

**ARE THE FOLLOWING INVOLVED?**

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>
| Surveys, questionnaires or interviews
   - If research is limited to use of surveys, questionnaires or interviews, Submit Exemption Application Form instead of this application. | ![ ] | ![ ] |
| Existing Patient Records and/or Specimens
   - If research is limited to study of existing medical records and/or samples, Submit Short Form instead of this application. | ![ ] | ![ ] |
| Investigational Drug(s) IND#
   - If "yes", do you intend to use the UNC Hospitals Investigational Drug Service? | ![ ] | ![ ] |
| Approved drugs for "non-FDA-approved" conditions | ![ ] | ![ ] |
| Placebo(s) | ![ ] | ![ ] |
| Experimental devices, instruments, machines IDE# | ![ ] | ![ ] |
| Genetic studies on subjects' specimens | ![ ] | ![ ] |
| Storage of subjects' specimens for future, as-yet-undesignated research
   - If "yes", see Instructions for Submitting IRB Applications for Research that Includes the Storage of Human Biologic Specimens. | ![ ] | ![ ] |
| Fetal tissue | ![ ] | ![ ] |
| Videotaping, audiotaping, filming of subjects | ![ ] | ![ ] |
| Non-patient volunteers | ![ ] | ![ ] |
| Patients as subjects | ![ ] | ![ ] |
| Minors (less that 18 years old)
   - If "yes", indicate: Age range to years | ![ ] | ![ ] |
| Do you intend to target your enrollment at:
   - Students or staff as subjects? | ![ ] | ![ ] |
|   - Non-English-speaking subjects? | ![ ] | ![ ] |
|   - Decisionally impaired or mentally incompetent subjects? | ![ ] | ![ ] |
|   - Prisoners, parolees and other convicted offenders as subjects? | ![ ] | ![ ] |
|   - Pregnant subjects? | ![ ] | ![ ] |
| Will HIV tests be performed? | ![ ] | ![ ] |
| Will subjects be studied at off-campus sites? | ![ ] | ![ ] |
| Is this a multicenter study?
   - If "yes", is UNC-CH the sponsor or coordinating center? | ![ ] | ![ ] |
| Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise
   - If "yes", approval by the Radiation Safety Committee is required. | ![ ] | ![ ] |
| Recombinant DNA or gene transfer to human subjects
   - If "yes", approval by the Biologic Safety Committee is required. | ![ ] | ![ ] |
| Is this an oncology study?
   - If "yes", submit this application directly to the Oncology Protocol Review Committee. | ![ ] | ![ ] |
| Will subjects be studied in the General Clinical Research Center?
   - If "yes", obtain GCRC Addendum from the GCRC and submit complete application (IRB application and Addendum) to the GCRC. | ![ ] | ![ ] |
III. Description of Proposed Research Activity

Entire application should not usually exceed 5 single-spaced pages using a 12-point font.

1. Purpose and Rationale: Provide a brief summary of the background information, state the research question(s), and tell why the study is needed. Avoid an extensive literature review.

A. Specific objectives

1) To determine the fractional uptake of ozone in the nose at rest and following moderate exercise in healthy young adults.

2) To determine the relationship between internal nasal physiology and nasal uptake of ozone before and after exercise in healthy young adults.

B. Background

The nose provides a first line of defense for inhaled gases and particles, especially during resting breathing when nasal breathing is most prevalent (1). Gases that are very water soluble, e.g. SO₂ and aldehydes, can be extracted in the nose by up to 95% during resting breathing. Reactive gases such as ozone may also be extracted by the nose to a lesser degree. Santiago et al (2) recently showed that fractional uptake of ozone decreased from 0.80 to 0.33 with increasing flow rate (range 3 to 15 L/min) when ozone was passed in one nostril and out the other while the subjects closed their velopharyngeal aperture. They also found a small decrease in fractional uptake with increasing ozone concentration (from 0.36 to 0.32 for concentrations of 0.1 to 0.4 ppm at a flow rate of 15 L/min). Theoretical considerations of nasal absorption of gaseous pollutants suggest that nasal geometry, especially surface area, should be an important predictor of nasal absorption of gaseous pollutants (1, 3). Recent studies document a wide range of human nasal geometry (4,5) using acoustic rhinometry (6). However, Santiago et al (2), in their limited number of subjects, found no correlations between parameters of ozone uptake and nasal volume measured by acoustic rhinometry. Besides diffusion across the air-mucus interface, ozone can also be lost by reaction with nitric oxide (NO) that is released into the airway lumen from the underlying tissue (7).

Exercise is known to naturally dilate the nasal airways (8) and mean nasal flow rates can reach values of 60 L/min during exercise (9). Production of NO in the nose has also been shown to decrease (10) with exercise. However, there is no information on the impact of exercise-induced physiologic changes on nasal uptake of ozone. Such information may be useful for assessing risk associated with ozone exposures during exercise and the variability of such risk in the population associated with human variability in nasal structure/physiology.

2. Subjects: Specify number, age, gender, ethnicity, and whether healthy volunteers or patients. If patients, specify the disease or condition and indicate how potential subjects will be identified. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided. NIH applications require that women, minorities, and children be included or that their exclusion be justified. If children are involved, refer to "Children as Research Subjects".

A group of thirty healthy adults, age 18-35, even mix of gender will be included in each group. Subjects will be recruited through local advertisements and scheduled for study by the principal investigator or clinical studies coordinator in the Center for Environmental Medicine. Though 30 subjects are required to complete the study, as many as 40 may be recruited to account for a portion of these subjects not being able to complete all phases of the study. All potential subjects will undergo a screening procedure, which includes medical history.

3. Inclusion/Exclusion criteria: List required characteristics of potential subjects, and those that preclude enrollment.

Exclusion criteria for participation will be:
a) Participants must be free of upper and lower respiratory infections for 4 weeks prior to study.

b) Due to potential complications from performing lung function tests, participants should have no history of ear, nose, or throat surgery over the last 5 years.

c) Participants should have no history of lung disease (asthma, fibrosis, or chronic obstruction), no history of chronic coughing and/or wheezing, and no history of nasal surgery or nasal obstructions.

d) Participants should have no history of cardiovascular disease or untreated hypertension.

e) Forced vital capacity (FVC) > 75% predicted (for height, weight, and sex) and the ratio of forced expiratory volume in 1 second to forced vital capacity (FEV1/FVC) must be greater than 70%.

f) No history of smoking within 5 years, and no history of greater than 0.2 pack-years prior to that.

g) No current, prolonged use, or use within 4 weeks of nasal steroids, antihistamines, or decongestants use of vitamin C, E and other antioxidants 24 hr prior to test.

h) No history of regular recreational drug use by nose or mouth inhalation.

4. Full description of the study design, methods and procedures: Include the type of experimental design; study procedures; sequential description of what will be asked of/done to subjects; assignment of subjects to various arms of the study if applicable; doses, frequency and route of administration of medication and other treatment if applicable; kinds of data to be collected; primary outcome measurements; and follow-up procedures. If the study involves treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls. This section (4) should generally not exceed 2 single-spaced pages using 12-point type.

Study day 1: Each subject will first undergo a screening, which includes a brief medical history, pulmonary function tests (spirometry and plethysmography), acoustic rhinometry, nasal NO production, and an exercise capacity test on a cycle ergometer. The measurements of airway resistance in the body plethysmograph will be made with the subject breathing through a mouthpiece and then through a nasal mask in order to measure total and nasal airway resistance to airflow. Following these tests a measure of each subject's predicted maximum exercise capacity on a cycle ergometer (9, 11) will be made. With heart rate monitored by 3 lead ECG, subjects will perform graded submaximal exercise at three increasing workloads (in watts) while maintaining a pedal rate of 60-70 revolutions/min. Each work load trial will last 5 minutes. The maximum of the three workloads will not exceed a heart rate of 170 beats/min. By linear extrapolation of the workload-heart rate relationship to each subject's age related predicted maximum heart rate (9, 11), the subject's maximum physical work capacity (PWCmax) will be determined. Acoustic rhinometry (Eccovision, Hood Laboratories) will be performed before and immediately after the bicycle exercise. This involves connecting a nosepiece to a subject's nose and sending a sound pulse from a wave tube through each nostril. The wave tube captures the "echo" and creates a map of internal nasal physiology by cross-sectional area. This test is non-invasive and takes less than 10 seconds to complete. Nasal NO production will also be measured before and immediately after the bicycle exercise (in each case after acoustic rhinometry) by connecting an NO analyzer sampling line to one nostril with the other nostril open to ambient air. During the measurements the subject will be asked to close the soft palate to prevent cross-contamination of samples. The sample collection methodology is quite similar to the method recommended by the European Respiratory Society (12). NO concentration in the sample will be measured with a model 270B nitric oxide analyzer (Siever Corp, Boulder, CO).

Study day 2: During each subject's second visit, we will make measurements of fractional uptake of ozone in the nose using a fast response ozone analyzer. The ozone at a concentration of 0.2 ppm will be generated by an
ozone calibrator (Photometric O3 Calibrator - Model 401, Advanced Pollution Instrumentation, Inc.) and stored in a 60L Tedlar bag for the experiments. Each subject will be fitted with a teflon nose piece connected to their nostrils and a teflon mouthpiece that will allow the ozone from the bag to be drawn through the nose and out of the mouth while the subject holds his/her breath for 10 seconds. Nasal uptake of ozone will be determined by comparing the ozone concentration entering the nose to that exiting the mouthpiece and will be measured for the subject's resting state and after moderate exercise on the bicycle ergometer (15 minutes at 40 – 60% PWCM). In each case (before and after exercise) three measurements of fractional uptake will be made at each of three flow rates through the nose (10, 20, and 30 L/min representing resting and light exercise flow rates). Acoustic rhinometry and nasal NO production (as described above) will also be performed before and after exercise and just prior to ozone uptake measurements. These measures will allow comparison between nasal uptake of ozone and nasal morphology/physiology before and after exercise. Results will allow us to interpret/determine the importance of these morphologic/physiologic variations on the filtering capacity of the nose for ozone.

5. Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable: include the number of required visits and approximate duration of each visit.

Each subject will visit the laboratory on two separate study days. The time associated with each visit is approximately 2 hours. These two visits will take place within a four-week period for each subject. The entire study should be completed within 9 months.

6. Where will the subjects be studied? If off UNC-CH campus, list locations.

These experiments will be conducted at the Clinical Exposure Facility of the United States Environmental Protection Agency, Clinical Research Branch. The facility has been described in detail in multiple protocols submitted within the last several years to the UNC Committee on the Protection of Rights of Human Subjects. These facilities are located at 104 Mason Farm Road, on the medical campus of the University of North Carolina.

7. Full description of risks and measures to minimize risks: Include risk of psychosocial harm (e.g. emotional distress, embarrassment, breach of confidentiality, etc.) economic harm (e.g. loss of insurability) and legal jeopardy (e.g. disclosure of illegal activity) as well as known side effects of study medication, if applicable, and risk of pain and physical injury.

Spirometry can potentially be associated with exacerbation of chronic ear or sinus conditions, particularly when surgery has been required recently, because of pressure changes in the nose (with nose-clip in place) that may occur during forced expiration. We will not study any subjects having a recurrent history of middle ear or sinus disease or those who have had head or neck surgery within the past 5 years.

The moderate exercise level for a short period of time (15 min) on the two study days will be scaled to each subject's fitness level. Subjects with any history of cardiovascular disease will not be allowed to participate in the study.

Risk to the subjects from ozone inhalation is minimal. The concentration of ozone, 0.2ppm, used in these experiments is comparable to ambient ozone levels measured during summer months in Chapel Hill. The cumulative time for ozone inhalation administered during the experiments is <5 min of exposure. At this ozone dose even the most sensitive individuals may experience only transient mild nasal irritation. These symptoms are fully reversible in < 1 hr and are without any sequela.

8. Benefits to subjects and/or society: The possibility of benefit to society should be clearly distinguished from the possibility of benefit to the individual subject, if any. If there is no direct benefit to the individual subject, say so. Do not list monetary payment as a benefit.

Adults will receive pulmonary function tests. They will have access to the results of these tests.
9. **Inducements for participation:** If monetary, specify the amount and how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it.

All subjects and potential subjects who complete the screening procedure will receive forty dollars when they complete it. In addition each participant will be paid $20/hour plus a $20 bonus for returning for the second visit, resulting in the following payment schedule:

<table>
<thead>
<tr>
<th>Visit one</th>
<th>$40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit two</td>
<td>$40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bonus</th>
</tr>
</thead>
<tbody>
<tr>
<td>$20</td>
</tr>
</tbody>
</table>

Thus, a total of $100 will be dispersed to each participant who completes the study. Subjects may withdraw at any time and receive the appropriate fraction of the total payment based on their participation up to that point.

10. **Costs to be borne by subjects:** Include clinic fees, diagnostic and laboratory studies, drugs, devices, transportation, all professional fees, etc. If there are no costs to subjects, indicate this.

No costs to be borne by participants.

11. **Statistical analysis:** If this is a single-center clinical trial, provide evidence that the sample size is sufficient to achieve the study aims and tell how the data will be analyzed. If a multicenter trial, indicate where and by whom statistical analysis will be performed.

Repeated measures comparisons will be used to statistically describe (1) pre vs. post exercise fractional ozone uptake, nasal structure (acoustic rhinometry), and nasal NO production, and (2) Ozone uptake for the 10, 20 and 30 L/min flow rates pre and post exercise. Multiple linear regression will be used to analyze relationships between fractional ozone uptake, nasal structure, and nasal NO production.

12. **Methods of recruiting:** Tell how prospective subjects are contacted. If they are UNC Hospital patients, the initial contact should be made by their treating physician, or by someone whom the patients know to have legitimate access to their medical records (for example, a clinical director). This may be accomplished by means of a letter from that individual to prospective subjects, requesting the patient’s permission to be contacted by the investigator.

Healthy adult subjects will be recruited through local advertising by the Center for Environmental Medicine, for which recruiting and screening protocols have been approved by the IRB. Experience from previous studies has shown that by further word-of-mouth we have been able to recruit more than sufficient numbers of healthy adults.

13. **How will informed consent be obtained?** Describe the process. When the consent of a legally authorized representative is substituted for consent of the adult subject, explain why this is necessary. If non-English-speaking subjects will be enrolled, a consent form should be prepared in their foreign language. Someone who is fluent in the subjects’ language must be available to interpret.

Consent will include a verbal discussion of the procedures to be explored, and a verbal review of the consent form for each study. Patients will be specifically warned of any potential risks, and will be informed that they will not be penalized in any way if they elect to withdraw from the study. The adult will be asked to read appropriate consent forms (appended), ask any questions they may have, and sign the forms if they feel they wish to participate. Two copies of each form will be signed so that one copy may be kept for the participants’ records. Consent will be acknowledged and documented with both the subjects written and dated signature on the consent form and a co-witness signature.
References


ADDENDUM B
University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Medical IRB Study #: 01-CEMLB-599
Consent Form Version Date: 7 Dec 2001

Title of Study: The relationship between internal nasal structure and nasal uptake of ozone in healthy adults before and after exercise
Principal Investigator: William D. Bennett, PhD
UNC-CH Department: Center for Environmental Medicine and Lung Biology
Phone number: 966-6229
Co-Investigators: Milan Hazucha, PhD; Todd Alter, MS, Keegan Musgrove-Wesley, BS, Philip Bromberg, MD
Sponsor: USEPA

You are being asked to take part in a research study. The investigators listed above are in charge of the study; other professional persons may help them or act for them.

What are some general things you should know about research studies?
Research studies are designed to gain scientific knowledge that may help other people in the future. You may or may not receive any direct benefit from participating. There may also be risks associated with participating in research studies.

Your participation is voluntary. You may refuse to participate, or may withdraw your consent to participate in any study at any time, and for any reason, without jeopardizing your future care at this institution or your relationship with your doctor. If you are a patient with an illness, you do not have to participate in research in order to receive treatment.

Details about this particular study are discussed below. It is important that you understand this information so that you can decide in a free and informed manner whether you want to participate. You will be given a copy of this consent form. You are urged to ask the investigators named above, or staff members who may assist them, any questions you have about this study at any time.
**What is the purpose of this study?**

When outside on the days with higher ozone levels, e.g., code orange or red, some individuals will experience breathing discomfort or may even cough. We do not know why some individuals will develop such respiratory symptoms while others will not. One thing that might affect this is how much ozone gets absorbed in our nose during breathing, which will decrease the amount that reaches the lung.

The purpose of this research project is:
1) to determine how much of the ozone present in the air we breathe will be absorbed in the nose when breathing at rest and during moderate exercise.
2) to determine the relationship between internal nasal structure and the amount of ozone that’s absorbed in the nose.

**How many subjects will participate in this study?**
If you decide to participate, you will be one of approximately forty adult subjects in this research study.

**How long will your participation last?**
Your participation in this study will last for approximately 1 month and consist of two visits. Each visit will take approximately two hours.

**What will happen if you take part in the study?**
During the course of this study, the following will occur:

1. You will be asked questions about your medical history, primarily whether you have had any chronic or acute lung and nasal problems.

2. During the first study visit you will receive instruction in and perform the following tests, 1) spirometry and 2) plethysmography, 3) an acoustic rhinometry test, 4) nasal nitric oxide measurement, and 5) a sub-maximal exercise test on a bicycle ergometer to determine your maximum work capacity. Spirometry requires inhalation of a deep breath followed by a rapid, forced exhalation. The measurements of airway resistance in the whole body plethysmograph will be made with you breathing through a mouthpiece and then through a nasal mask in order to measure total and nasal airway resistance to airflow. The bicycle ergometer test will require you to ride a stationary exercise bike for no longer than 15 minutes while we monitor your heart rate and workload as we gradually increase the difficulty of pedaling. Before and after exercising we will measure the internal structure of your nose, by attaching a nosepiece to your nose and passing sound waves into your nose. This measure, referred to as acoustic rhinometry, is non-invasive and will not cause any discomfort to you. Also before and after exercise we will measure how much nitric oxide is produced in your nose by attaching a sampling line to one nostril with the other nostril open to room air.

3. On the second visit, within a month of the first, you will have the following tests done on you: (1) Acoustic Rhinometry, (2) Nasal Nitric Oxide production, (3) Ozone absorption in the nose, and
(4) Exercise. Nasal ozone absorption requires a concentration of ozone similar to what you might breathe outdoors to be passed through your nose via a nosepiece and out of your mouth while you hold your breath for 10 seconds. This procedure will occur twice, before and immediately after exercising. Each time 9-12 repeat measurements will be made. Before each series of measurements of ozone absorption (before and after exercise) both acoustic rhinometry and nasal nitric oxide production will be measured. Bicycle exercise will last no longer than 15 minutes at 40-60% of your maximum work capacity, as determined from the first visit. This second part of the study should take approximately 2 hours.

**Are there any reasons you should not participate?**
You should not participate in this study if you have any history of lung or cardiovascular disease, chronic cough or wheeze, smoking history, nasal obstructions or nasal surgery, recurrent middle ear or sinus disease, and/or have had an upper or lower respiratory infection during the 4 weeks prior to study.

**What are the possible risks or discomforts?**
This study might involve the following risks and/or discomforts to you:

1. Risk to you from ozone inhalation is minimal. The total ozone dose you will inhale during the study amounts to 5 min of breathing Chapel Hill air during code orange or red days in the summer. At this ozone dose even the most sensitive individuals may experience only mild transient throat irritation and cough. These symptoms are fully reversible in less than 1 hr and are without any lasting detrimental effects.

2. All exercise will be at sub-maximal levels and be for a short period of time. You will be monitored throughout the exercise protocol and may cease exercising at any time you wish.

3. Pulmonary function tests are of little or no risk to you. Some people may cough or feel light-headed when performing spirometry. If this happens, it should go away very quickly. Spirometry might also exacerbate chronic ear or sinus conditions, particularly when surgery has been required recently, because of pressure changes in the nose (with nose-clip in place) that may occur during forced expiration.

**What are the possible benefits?**
The study itself will have no direct benefit to you. If you wish you may be provided with the results of all lung function tests to share with your doctor. We hope to learn things from this research that will help us understand how the nose and lungs work.

**What if we learn about new risks during the study?**
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.
How will your privacy be protected?
No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-CH will take all steps allowable by law to protect the privacy of personal information. Records will be secured within locked file cabinets in the laboratory of the principal investigator and subjects will be identified within these files and computer files by ID numbers.

Will you be paid for participating?
You will receive $40 upon completion of the first visit (screening and pulmonary function testing). You will receive $60 for the second visit. Thus for completion of the whole study you will receive $100. Parking will be provided free of charge on the parking deck.

Will it cost you anything to participate?
There will be no costs to you for participating.

Who is sponsoring the research?
This research is funded by US Environmental Protection Agency. This means that the research team is being compensated by the sponsor for conducting the study. The researchers do not, however, hold a direct financial interest in the sponsor or in the product being studied.

What will happen if you are injured by this research?
In the event of personal injury resulting directly from the research procedures, financial compensation cannot be provided by The University of North Carolina at Chapel Hill. All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study. If such complications arise, the researchers will assist you in obtaining appropriate medical treatment but The University of North Carolina at Chapel Hill does not provide financial assistance for medical or other costs. You do not waive any liability rights for personal injury by signing this form.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have further questions, or if a research-related injury occurs, you should call William D. Bennett, PhD at 966-6229.

What if you have questions about your rights as a subject?
This research has been reviewed and approved by the Committee on the Protection of the Rights of Human Subjects (Medical IRB) at the University of North Carolina at Chapel Hill. If you have any questions or concerns regarding your rights as a research subject, you may contact the Chairman of the Committee at (919) 966-1344.

Subject's Agreement:
I have read the information provided above. I voluntarily agree to participate in this study.

________________________________________________________________________________________
Signature of Research Subject ___________________________________________________________
Date

________________________________________________________________________________________
Printed Name of Research Subject _______________________________________________________

________________________________________________________________________________________
Signature of Person Obtaining Consent ___________________________________________________
Date

________________________________________________________________________________________
Printed Name of Person Obtaining Consent _______________________________________________