THE HEALTH IMPACT OF NITRIC OXIDE: EFFECTS ON LUNG FUNCTION AND CELLULAR AND BIOCHEMICAL PROCESSES IN HEALTHY HUMANS

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ABSTRACT

Epidemiological studies have reported associations between ambient concentrations of nitrogen dioxide (NO_2) and morbidity, but human exposure studies with ambient concentrations of NO_2 have not shown significant effects. Since a significant concentration of nitric oxide (NO) is often present in ambient air, the epidemiological findings attributed to NO_2 could be due to NO. This literature survey addressed whether an ambient air quality standard for NO was warranted, and/or whether there was evidence that inhalation of ambient NO has health effects that should be investigated further. While it is clear that NO is biologically active throughout the body, and that ambient NO could induce health effects, the review concludes that currently there is insufficient evidence to warrant an ambient air quality standard for NO. The review concludes that NO can be both beneficial and injurious, and suggests that ambient concentrations of NO could affect cardiopulmonary regulation, pulmonary inflammation, asthma and other inflammatory lung diseases, host defense, immune responses and platelet function. Suggested areas for further research include: (1) biochemical and/or cellular changes in the lungs indicative of pulmonary inflammation; (2) effects on asthma and other inflammatory diseases; (3) whether environmental NO is beneficial; (4) effects on platelet function; (5) effects on endogenous NO production; (6) effect on ventilation/perfusion (V_A/Q) mismatching; and (7) the role of NO in epidemiological findings attributed to NO_2, carbon monoxide (CO) and particulate matter (PM).

EXECUTIVE SUMMARY

Background: The objective of this paper was to critically review literature on nitric oxide (NO) to determine whether there are sufficient data to suggest that exposure to ambient concentrations of NO causes adverse health effects. Epidemiological studies have reported associations between ambient concentrations of nitrogen dioxide (NO_2) and morbidity, including incidence of respiratory infections, croup, exacerbation of asthma, bronchitis, ischemic cardiac and cerebrovascular diseases at NO_2 concentrations commonly measured in ambient air. However, human exposure studies conducted with ambient concentrations of NO_2 alone have not shown significant effects, even at concentrations higher than typically observed in ambient air. This, along with the finding that there can be a significant concentration of nitric oxide (NO) in ambient air with a similar concentration profile to NO_2, has raised the question as to whether the epidemiological findings attributed to NO_2 exposure may actually be due to the precursor, NO. This possibility is particularly intriguing in light of recent findings about the biological significance of NO.

Methods: The review focuses on a selection of recent literature in the areas of basic and clinical sciences and epidemiology, which gives insight into the potential interactions between inhaled ambient nitric oxide and endogenous NO production. Several questions guided the development of this paper:

1) Are ambient concentrations of NO harmful?
2) Is there an association between increased exposure to NO and respiratory illness, for example, asthma, chronic bronchitis, chronic obstructive pulmonary disease, or respiratory infections?
3) What targets or markers should be examined in studies of the health effects of NO?
4) Does inhaled ambient NO induce or modulate endogenous production of NO?

Results: The explosion of information about the ubiquity and wide-ranging activity of NO in the body clearly suggests that early studies that concluded NO inhalation had no significant effects on human health did not examine the correct endpoints. There is little basic biological or clinical literature available that directly addresses issues critical to assessment of possible health effects from exposure to ambient NO. The literature reviewed indicates that NO can be both beneficial and injurious, and suggests that even the comparatively low concentrations of NO in the ambient air could affect cardiopulmonary regulation, pulmonary inflammation, asthma, and other inflammatory lung diseases. There is also evidence suggestive of possible effects on host defense, cell-mediated immune responses and platelet function.

Conclusions: While it is clear that NO is biologically active throughout the body, and that ambient NO could, at least theoretically, affect endogenous NO production and/or induce health effects, there is insufficient evidence available at this time to warrant consideration of an ambient air quality standard for NO. The

literature reviewed suggests several avenues for future research relevant to air quality health effects and regulation. Suggested areas for further research include: (1) biochemical and/or cellular changes in the lungs indicative of pulmonary inflammation; (2) effects on asthma and other inflammatory diseases; (3) whether environmental NO is beneficial; (4) effects on platelet function; (5) effects on endogenous NO production; (6) effect on ventilation/perfusion (V_A/Q) mismatching; and (7) the role of NO in epidemiological findings attributed to NO_2, carbon monoxide (CO) and particulate matter (PM).

INTRODUCTION

Epidemiological studies have reported associations between ambient concentrations of nitrogen dioxide (NO_2) and morbidity, including incidence of respiratory infections, croup (Rebman et al., 1991), exacerbation of asthma (Pönka et al., 1991), bronchitis (Raaschow-Nielsen et al., 1995), ischemic cardiac and cerebrovascular diseases (Pönka et al., 1996) at NO_2 concentrations commonly measured in ambient air. However, human exposure studies (Adams et al., 1987; Blomberg et al., 1997; Drechsler-Parks et al., 1989; Folinsbee et al., 1978; Solomon et al., 2000) conducted with ambient concentrations of NO_2 alone have not shown substantial effects, even at concentrations higher than typically observed in ambient air. This, along with the finding that there can be a significant concentration of nitric oxide (NO) in ambient air with a similar concentration profile to NO_2, has raised the question as to whether the epidemiological findings attributed to NO_2 exposure may actually be due to the precursor, NO. This possibility is particularly intriguing in light of recent findings about the biological significance of NO.

Pönkä et al. (1991) analyzed the relationship between hospital admissions for asthma and ambient concentrations of NO_2, NO, sulfur dioxide (SO_2), carbon monoxide (CO), ozone (O_3) and total suspended particles. Regression analysis revealed that NO and O_3 were most strongly correlated with asthma. Among children, O_3 and NO were significantly correlated with hospital admissions. In a later study (1996), the same authors reported that hospital admissions for ischemic cardiac disease were associated with ambient concentrations of NO and O_3, and that those due to cerebrovascular disease were associated with NO_2.

Several other European studies have found statistically significant correlations between NO and NO_2 and respiratory illness (Rebman et al., 1991; Raaschou-Nielsen et al., 1995). Raaschou-Nielsen et al. (1995) reported a high prevalence of chronic bronchitis and asthma in Copenhagen street cleaners correlated with ambient NO, NO_2, CO, SO_2 and O_3. However, since ambient concentrations of these air pollutants are highly correlated, it is difficult to determine the effects of each individually.

Peters et al. (1999) conducted a 10-year study of respiratory health and air pollution exposure in public school children in 12 Southern California communities with a wide range of air quality. Repeated pulmonary function tests were completed on 3292 children. They found an association between concentrations of several air pollutants and reduced pulmonary function in female subjects. Reduced expiratory airflow rates were significantly associated with PM10, PM2.5 and NO_2. NO_2 was most strongly associated with reduced FVC, although it was also significantly correlated with reduced forced expiratory volume in one second ($FEV_{1.0}$) and maximum mid-expiratory flow rate (MMEF). However, as pointed out by the authors, this cross-sectional survey did not

distinguish between acute reversible effects of air pollutants and chronic effects, nor did it account for the possibility of interaction between pollutants, or for reactions among the chemical species present in the ambient air.

There are no studies of human exposure to NO similar to those that have been reported for ambient concentrations of NO_2 exposure. Controlled studies on human subjects exposed to ambient concentrations of NO_2 have found minimal to mild effects on pulmonary function, airways reactivity and lung inflammation (Adams et al., 1987; Blomberg et al., 1997; Drechsler-Parks et al., 1989; Folinsbee et al., 1978; Solomon et al., 2000). Drechsler-Parks (1995) reported that NO_2 exposure inhibited the increase in cardiac output with exercise in healthy older adults, although this finding has not been confirmed. Exposure to very high concentrations of both NO and NO_2 can lead to respiratory irritation, chest pain, and pulmonary edema (Frawley et al., 1964; Troncy et al., 1997). However, NO is about one-fifth as potent an oxidant as NO_2.

Since the biological significance of NO has become clear, the question has arisen as to whether or not effects attributed to NO_2 in epidemiology studies that are not duplicated in human exposure studies are in fact related to the precursor, NO. Further, since NO has such wide-ranging endogenous activity in the body, if inhalation of ambient NO is capable of altering physiological function, effects could theoretically occur throughout the body, rather than just in the lungs.

Purpose

The objective of this paper was to critically review literature on nitric oxide (NO) to determine whether there are sufficient data to suggest that exposure to ambient concentrations of NO causes adverse health effects. Given that there are over 40,000 papers listed in Medline (as of April 2001) containing the key word nitric oxide, it was impossible to review everything that has been published on this topic. Therefore, the review focused on a selection of recent literature in the areas of basic and clinical sciences and epidemiology, which gives insight into the potential interactions between inhaled ambient nitric oxide and endogenous NO production.

Several questions guided the development of this paper:

1) Are ambient concentrations of NO harmful?
2) Is there an association between increased exposure to NO and respiratory illness, for example, asthma, chronic bronchitis, chronic obstructive pulmonary disease, or respiratory infections?
3) What targets or markers should be examined in studies of the health effects of NO?
4) Does inhaled ambient NO induce or modulate endogenous production of NO?
5) Does “ambient” NO increase in a hospital setting where NO is being used clinically?

BACKGROUND

Nitric oxide (NO) is a colorless, odorless gas at ambient temperature. Because it is only slightly soluble in water (73.4 ml/l), NO penetrates deeply into the lungs. It is very soluble in lipids, thus it can easily diffuse across cell membranes. It has a density approximately equal to air (1.04), and chemically, is a free radical. NO reacts in water to form nitrate and nitrite. As an oxidant, NO is $1/5^{th}$ as potent as NO_2, and $1/25^{th}$ to $1/50^{th}$ as potent as O_3 (Troncy et al., 1997).

Oxides of nitrogen (NO_X) occur naturally in the atmosphere as a result of biogenic processes (e.g., microbial processes in soil), biomass burning, lightning, etc. (Thakur et al., 1999) On a global scale, anthropogenic sources of NO_X are comparable to or exceed the natural production (IPCC, 1995). In urban areas however, anthropogenic emissions (primarily from the combustion of fossil fuels and mostly from motor vehicles in California (CARB, 2001) are much greater than emissions from natural sources (Mδller, 1992). Ambient NO concentrations in urban areas are typically 2 to 3 orders of magnitude higher than in rural and remote areas, approximately 20 ppb vs. 0.2 ppb vs. 0.02 ppb (National Research Council, 1991).

Historically, investigations into the potential health effects of NO have been limited because NO_X emissions, which are primarily in the form of NO, are rapidly converted to nitrogen dioxide (NO_2) and other oxidized nitrogenous compounds in the atmosphere (Finlayson-Pitts and Pitts, 2000). Photochemical processes rapidly oxidize ambient NO during the day and ozone titration (the instantaneous reaction of O_3 with NO) oxidizes NO day and night (Seinfeld and Pandis, 1998). In urban areas where fresh NO emissions have totally titrated the O_3 present in the air, NO concentrations can increase and be significant for multiple hours, particularly near heavily traveled roadways. Furthermore, people working/living/cooking near combustion sources will be exposed to more NO than the average person. A closer look at ambient and indoor concentrations of NO is warranted to estimate the exposure of various groups to NO.

AMBIENT NO

Ambient (outdoor) hourly-averaged NO concentrations in California vary widely, ranging in 1999 from 0 to 873 ppb. For comparison, maximum hourly NO concentrations observed elsewhere in North America in 1999 were 500 ppb in Ohio, 654 ppb in Texas, 679 ppb in Illinois, 738 ppb in New York, 789 ppb in Colorado, and 957 ppb (based on incomplete data) in Mexico (USEPA, AIRS). The bulk (>95%) of the world's anthropogenic NO_X emissions from fuel combustion occur in the Northern Hemisphere (IPCC, 1995). Ambient NO concentrations, nearly as high as in North America (600 ppb), have been reported in Finland (Kharitonov et al., 1997).

The highest NO concentrations in California typically occur at the Lynwood monitoring site in the Los Angeles urban area. Figure 1 illustrates the average diurnal, day-of-week, and seasonal variations in NO concentrations at the Lynwood site. As the profiles illustrate, the exposure of people to NO is strongly associated with driving patterns (e.g., morning commute) and the stronger and more frequent ground-based temperature inversions of winter which trap the emissions near ground level.

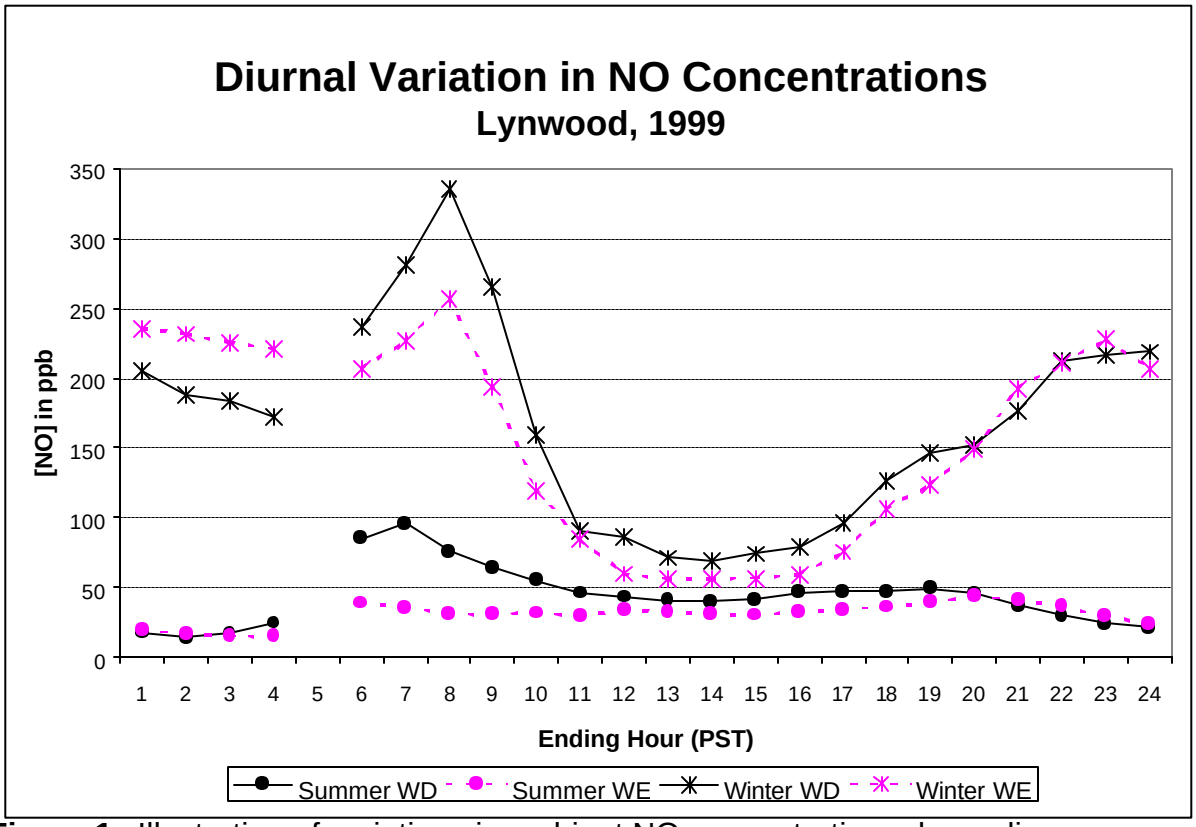


Figure 1. Illustration of variations in ambient NO concentrations depending on the time of day, the day of the week, and the time of year.

INDOOR NO

Although there is little peer-reviewed literature on the subject, it appears that indoor NO is largely derived from outdoor air which enters a building either through natural ventilation, or leakage. Indoor concentrations tend to parallel outdoor concentrations, although indoor concentrations are elevated relative to outdoor concentrations when indoor combustion appliances are used. Indoor emissions of NO are derived from natural gas fired cooking stoves, space heaters and gas fireplace lighters and logs. Small amounts of NO are emitted by kerosene space heaters. Wood combustion, on the other hand, does not appear

to be a significant source of indoor NO exposure (Rowe et al., 1991). Weschler et al. (1994) reported that indoor NO concentration has a strongly diurnal cycle, with peaks corresponding to periods of indoor combustion appliance use. These authors reported indoor concentrations ranging from 1 to 386 ppb for the period of July 1992 through August 1993 in a home in Burbank, CA.

There is little information available on indoor NO concentrations. Phillips et al. (1993) reported that NO concentrations inside four offices in England, although lower than outdoor concentrations, followed a similar pattern to the outdoor NO concentration profile. The measured concentrations in the four offices ranged from not detectable to 19 ppb.

Mourgeon et al (1997) monitored indoor and outdoor concentrations of NO in an Intensive Care Unit (ICU) in which NO was frequently administered to patients. They found that the concentration of NO in the ICU correlated mainly with outdoor concentrations, and that it did not increase during periods when NO was being administered to patients. Williams et al. (1995) reported that concentrations of NO in ICU's were approximately 1/100 of the OSHA standard of 5 ppm. They suggested that given these findings, it was probably not necessary to vent all exhaled gases from the room, since NO did not appear to accumulate in the air.

In contrast, Rowe et al. (1991) reported that indoor concentrations of NO of 30 to 50 ppb were typical at several sites in Riyadh, Saudi Arabia. He also reported a peak indoor NO concentration of about 500 ppb. This concentration range was significantly higher than the 2 to 3 ppb reported by the same authors for outdoor air in the same region. It is unclear why the concentrations reported by Rowe et al. are so different from those reported by Phillips et al. (1993), and the California Air Resources Board. The paper does not provide information on monitoring methods, time of day at which monitoring occurred, or what time unit the measurements cover (i.e., hourly or daily average).

We sampled NO concentration in our hospital laboratory during two separate one-month periods: April 1-29, 1999, and July 20 to August 20, 1999. Measurements were recorded two to four times per day using a Sievers NOA medical-grade NO analyzer. This analyzer uses a chemiluminescence technique that has been extensively validated for human use. The instrument has a sensitivity of <1 ppb, and a range of <1 ppb to 500 ppm, and is linear over the entire range, so a two-point calibration can be performed anywhere along the range. We found that all measurements (except one) made in our laboratory were less than 15 ppb, and that 84% of measurements were less than 6 ppb. These concentrations are in the range reported by Phillips et al (1993).

NO MEASUREMENT

Although not usually recorded, NO is directly measured by the chemiluminescent analyzers typically used to monitor NO_2. These analyzers also measure NOx, and then subtract the NO value from the NO_X value, yielding the NO_2 concentration. In the clinical arena, NO and NO_2 are also typically measured by a chemiluminescent analyzer. Electrochemical monitors are also available. Moutafis et al. (1995) reported that electrochemical methods are comparable to chemiluminescence, but are more expensive. The chemiluminescence method is considered more practical for clinical use. Etches et al. (1995) compared the two monitoring techniques, and came to similar conclusions as Moutafis et al. (1995).

Frawley and Tibbals (1997) compared three NO monitors during mechanical ventilation in a pediatric ICU. They concluded that electrochemical monitors may be used to guard against potentially toxic concentrations, i.e., concentrations greater than 20 ppm. However, they expressed a lack of confidence in the ability of the electrochemical monitors to regulate NO at low clinical levels, i.e., less than 5 ppm.

Monitoring Methods & Approaches

Electrochemical Sensors

An electrochemical sensor cell contains an aqueous electrolyte (e.g. diluted sulfuric acid) sealed with a hydrophobic gas permeable membrane. Two to three electrodes are located inside the sensor, surrounded by electrolyte. The electrode surfaces are coated with special catalyst-materials (e.g. gold or platinum). When a chemical reactive gas enters the sensor through the membrane, the gas molecule is either oxidized or reduced at the catalytic surface of the measuring electrode; the chemical process releases electrons causing a current flow between the measuring and the counter electrode. That current is proportional to the gas concentration.

A sensor can be made selective to a certain gas, by using the right combination of electrolyte, electrode material and bias voltage. The sensor bias, voltage (mV range) applied between the measuring and reference electrode, is critical. A variation of the bias controls the sensor sensitivity to a certain gas. A reference electrode and operational amplifier are required to maintain constant bias during a current flow. This is because current flow causes a voltage drop when the sensor is exposed to the gas. The "static potential circuitry" improves the linearity of an electrochemical sensor.

Electrochemical reactions are temperature-dependent; it is thus necessary to compensate for these effects. To design sensors with a linear output signal in a wide temperature range, an electrochemical sensor should therefore feature an

embedded temperature sensor. The reaction processes inside a nitric oxide (NO) sensor are provided as an example of the process:

At the measuring electrode, in "electrode-solution-interface" (gas, catalytic measuring electrode, electrolyte), the nitric oxide is oxidized.
Reaction at the measuring electrode:

$$NO + H_2O \rightarrow NO_2 + 2\,H^+ + 2\,e^-$$

Reaction at the counter electrode:

$$O_2 + 4\,H + 4\,e^- \rightarrow 2\,H_2O$$

Through NO oxidation and O_2 reduction, two electrons per molecule of NO are released. This results in a current flow of approximately 0.1 milli-Amps per ppm NO.

ARB has approved and used such a sensor for NO_2 and nitrous oxide (HONO) monitoring for indoor applications (Kelly & Myers, 1999). The dual channel sensor set up in this program proved remarkably stable and useful. Electrochemical sensors however suffer from electrolyte and sensor deterioration and loss of efficiency. Such sensors have to be continuously calibrated and quality assured and occasionally replaced.

Chemiluminescence Analysis

NO is sampled through the inlet and mixed with ozone, a gas-phase reaction occurs that produces a characteristic luminescence with an intensity that is linearly proportional to the concentration of NO. A photomultiplier tube senses the luminescence generated by the reaction. To measure other nitrogen species, they are first reduced to NO with a molybdenum converter heated to 325° C and then reporting the total concentrations as NO_X. The analyzer switches between measuring NO and NO_x and electronically computes the difference between NO_X and NO. The difference is mostly NO_2 as the other major constituent of NO_X. The instrument's converter can also convert other nitrogenous species, such as nitric acid and peroxyacetyl nitrate (PAN), to NO. Nitric acid and nitrate particles can be removed from the sample by installing a nylon filter on the sample inlet.

The calibration standards consist of a dilution flow metering system, NO/NO_X-free dilution air (zero air) system, and a cylinder of compressed gas containing a known amount of NO. The manually operated dilution system contains one flow controller (mass or volumetric) to meter accurate amounts of span gas, a second flow controller (mass or volumetric) to meter accurate amounts of dilution air, and a Teflon-lined or glass mixing chamber. To generate dilution air, ambient air is forced through desiccant, Purafil, and activated charcoal. Purafil (potassium permanganate) oxidizes NO to NO_2 that is then removed by the charcoal. A cylinder of compressed gas provides a source of approximately 50 ppm NO in a balance of nitrogen. The dilution system also has a section that produces a known

concentration of NO_2 by performing a gas phase titration (GPT) in which O_3 is mixed with NO to generate NO_2.

Zero and up to five upscale concentrations of NO are introduced to the instrument. The concentrations of NO range from 10% to 90% of the analyzer range with one near the span point of 450 ppb and one near the precision point of 100 ppb. Delivery to the analyzer is through as much sample line as possible including the switching solenoid valve and any inline filters.

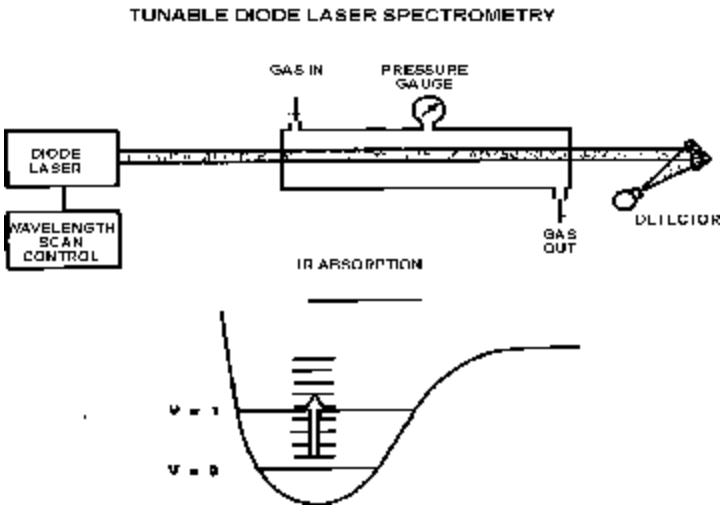
This is the approved EPA method for ambient monitoring of nitrogen species. Over a quarter century NO and total oxides of nitrogen data have been collected using this method. Quality assurance and sampling methodology for this method has continually evolved and although NO_2 is probably not entirely measured correctly; chemiluminescence is the most durable method for measuring NO (Parrish & Fehsenfeld, 2000).

Laser Induced Fluorescence (LIF) & Tunable Diode Laser Absorption Spectroscopy (TDLAS)

Laser Induced Fluorescence (LIF) relies on exciting the target NO molecule with sufficient light energy through a laser (usually a diode pumped YAG (Yttrium Argon Gallium) laser that pumps a tunable dye laser). Upon returning to the steady state, the excited NO molecule releases the energy in frequency specific fluorescence that is detected by a photocell. This then produces a current proportional to the concentration of NO. This approach has proven very successful in combustion analyses of NO (Malmsten and Axner, 1996).

$NO + h< \text{(at excitation frequency)} \rightarrow NO^*$
$NO^* \rightarrow NO + h< \text{(at fluorescence frequency)}$

Tunable Diode Laser Absorption Spectroscopy (TDLAS) also relies on directing specific frequency laser at the NO molecule, but this time it is intended that absorption rather than fluorescence to occur. The NO sample is drawn into the cell at reduced pressure. Most frequently, a multi pass absorption cell based and a base paths of 0.3 to 1.5 meters are used. Such cells result in total absorption path lengths ranging between 10 and 200 meters. The transmitted light is recorded with a solid state detector as the laser wavelength is repetitively scanned through absorption features of the NO. Wavelength scanning is generally accomplished by applying a ramp signal (typically a saw tooth ramp) to the quiescent injection current at frequencies of 10 - 100 Hz. Quantitative information is obtained using the Beer-Lambert Law. We use transmitted intensity at line center, measurements of pressure, path length, and absorption cross section to obtain a concentration.



LIF and chemiluminescence tend to agree well even in very low concentrations (ppt) (Parrish & Fehsenfeld, 2000). TDLAS may have limit of detection problems (Parrish & Fehsenfeld, 2000).

BASIC BIOLOGY

Over the past 10 to 15 years biological research has shown that NO is produced endogenously by most cell types, including, but not limited to, bronchial epithelial cells, vascular endothelial cells, various types of leukocytes, platelets, nerves, and smooth muscle cells. NO has been shown to be involved in many cellular and regulatory processes, including bronchodilation, vasodilation, nerve conduction, promotion of enzyme reactions, activation of leukocytes, maintenance of sterility in the lungs, and release of various cytokines and other immune reactants. It has also been shown to be involved in mediating inflammation.

Because NO is a very small, lipid soluble molecule, it passes through cell membranes. Once inside a cell, it interacts directly with intracellular proteins, in contrast to the majority of “extracellular” signaling molecules that mediate their effects by binding to cell surface receptors (Curran, 1996). NO differs from a “classical” mediator in that it is a very simple molecule which acts as a covalently reactive redox-type mediator, contrary to classical mediators, which are typically complex molecules that depend on a “fit” with a specific receptor for action.

In vivo, NO is synthesized from the terminal guanidine nitrogen of L-arginine when it is converted to L-citrulline by the activity of the enzyme nitric oxide synthase (NOS). Two categories of NOS have been described: constitutive (cNOS) and inducible NOS (iNOS). NOS isoforms are activated at the cell surface by either a second messenger, i.e. Ca++ (cNOS), or by gene transcription (iNOS). The isoforms of NOS are hemoproteins with structural similarities to cytochrome P450 (Steudal et al., 1999).

Constitutive NOS (cNOS) is expressed by endothelial, neuronal and other cells, and is involved in regulation of systemic blood pressure, regulation of blood flow via its effects on blood vessel caliber, neurotransmission in the brain, penile erection, and regulation of gastrointestinal function. It is tightly regulated, is calcium- and calmodulin-dependent, and releases picomoles of NO within a short time in response to receptor stimulation by agonists that induce opening of membrane Ca^{++} channels. NO production begins when Ca^{++} entering the cell reversibly binds to calmodulin. cNOS is present in cells in a preformed state, therefore its release can be started and stopped quickly (Curran, 1996). Because the increase in intracellular calcium is usually transient, the NO activity produced by the cNOS isoforms is generally local and short-lived. These forms of NOS probably function mainly as signaling molecules (Flak and Goldman, 1996). Constitutive NOS in nerve cells, nNOS, seems to be associated with nonadrenergic/noncholinergic (NANC) neurons, where it functions as a neurotransmitter. Constitutive NOS in the endothelial cells, eNOS, is increased by acetylcholine, bradykinin, serotonin and other mediator release. eNOS activity modulates systemic and pulmonary vascular tone, and plays a role in lung development and disease (Steudal et al., 1999).

Inducible NOS (iNOS) is expressed by endothelial and epithelial cells, smooth muscle cells, and immune cells (i.e., macrophages, neutrophils, mast cells). It is calcium and calmodulin independent, is activated by gene transcription, and produces more NO for a longer period of time than any isoform of cNOS. For example, the amount of NO released by a fully activated macrophage is 1000 times that released by cNOS activation of an endothelial cell. In contrast to cNOS, iNOS is produced *de novo*, and thus its induction is not immediate, and its production can continue for a prolonged period of time (Curran, 1996). It is regulated at the transcription level after induction by any of a number of different stimuli, including lipopolysaccharide and oxidized low-density lipoproteins, and by such cytokines as interferon-γ (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin 1-beta (IL-1β). It should be noted that these substances are promoters of immune and inflammatory reactions. Thus, iNOS appears to play a role in inflammatory and immune responses (Flak and Goldman, 1996; Steudal et al., 1999). iNOS may also be induced by airway cells in response to environmental pollutants and/or common environmental allergens. Treatment with a combination of lipopolysaccharide, IFN-γ, TNF-α and IL-1β is particularly effective in increasing induction of iNOS. Singly, these inflammatory mediators are not sufficient for induction of iNOS production (Curran, 1996; Gaston et al., 1994; Steudal et al., 1999).

Most cell types studied appear capable of producing NO, although the major *in vivo* source appears to be epithelial cells. Although epithelial cells produce both types of NOS, iNOS expression usually predominates (Asano et al., 1994; Guo et al., 1995; Watkins et al., 1997). Regulation of iNOS appears to be both cell- and mediator-specific, acting at the mRNA transcriptional level, as well as later in the

process of translation and protein synthesis (Lorsbach et al., 1993; Vodovotz et al., 19913; Tsutsumi et al., 1999).

Release of NO, regardless of which form of NOS is involved, leads to stimulation of cyclic GMP (cGMP) synthesis. The NO released through cNOS activation has local discrete actions that are exerted via activation of the enzyme guanylate cyclase in the target cell (Gustafsson, 1993). Soluble guanylate cyclase (sGC) mediates NO effects by converting guanosine 5'triphosphate to cGMP, which is found in the cytosol of almost all cells. sGC is a heme-containing protein with α and β subunits. The heme portion of the molecule is essential for enzyme activation. sGC is hydrolyzed to GMP, which is the physiological mechanism of inactivation of cGMP (Gaston et al., 1994).

Once NO is produced, regardless of the induction method, one of three things happens: (1) it is inactivated by reacting with hemoglobin or albumin, (2) it acts as a biological mediator via its effect on guanylate cyclase, or (3) it forms toxic radical derivatives, i.e. peroxynitrates.

NO is inactivated by reaction with target molecules, its half-life being only a few seconds. Systemically, NO binds to hemoglobin, thereby inactivating it and resulting, in the presence of oxygen, in formation of methemoglobin (MetHb). MetHb is subsequently reduced to ferrous Hb by MetHb reductase and other enzymes present in the red blood cells. As long as activity of MetHb reductase is adequate, there is no significant increase in MetHb with NO exposure (Ewetz, 1993). *In vitro* data suggests that the affinity of hemoglobin for NO is several thousand times higher than for carbon monoxide (Ewetz, 1993). Gustafsson (1993), on the other hand, reported that the affinity of NO for Hb is 200-fold higher than carbon monoxide. NO can also bind with the sulfhydroxyl group of hemoglobin to form S-nitrosohemoglobin. This may be a mechanism for transport of NO through the peripheral circulation. S-nitrosohemoglobin is converted to nitrate and nitrite, which are eliminated in the urine with a half-life of 5-8 hours. Another metabolic pathway for inactivation of NO involves binding to superoxide, with subsequent release of nitrate (NO_3^-). NO is also inactivated by binding to thiols. Biotransformation of nitrate and nitrate derived from inhaled NO follows the same pathways as nitrate and nitrite derived from food. Almost 70% of inhaled NO appears in urine within 48 hours as nitrate (NO_3^-). The remaining 30% of inhaled NO is secreted via the salivary glands as nitrite (NO_2^-). A small amount of inhaled NO is converted to NO_2 gas in the stomach. Some NO_2^- in the intestines is reduced to ammonia, reabsorbed and converted to urea (Yoshida et al., 1983, 1987).

PHYSIOLOGICAL EFFECTS OF NO

Cardiopulmonary System

Pulmonary vascular tone in human airways is regulated through the NANC nervous system by continuous production of NO, which inhibits bronchoconstriction, rather than inducing bronchodilation (Steudal et al., 1999). Reduction in NO production in the lung leads to pulmonary vasoconstriction, and mismatching of alveolar ventilation (V_A) and cardiac output (Q) (Änggård, 1994; Singh and Evans, 1997). Improvement in V_A/Q matching by NO-mediated bronchodilation and pulmonary vasodilation is primarily accomplished by the NO effect being concentrated in the most constricted airways and blood vessels. The ultimate effect is improved V_A/Q matching, and improved oxygenation of the blood. The bronchodilator action of inhaled NO seems to be weaker in asthmatics than the commonly used β_2- adrenergic agonists, and appears to provide no significant benefit to patients with chronic obstructive pulmonary disease (COPD) (Högman et al., 1993; Takahashi et al., 1998).

Resting vascular tone is regulated by endothelial NO production in the small arteries and arterioles. The veins synthesize little NO under basal conditions. In the cardiovascular system, diminished NO production leads to increased vascular tone, vasospasm, and thrombosis related to enhanced platelet and white blood cell adhesion to the blood vessel walls (Singh and Evans, 1997). Over-production of NO is thought to contribute to vascular hyporeactivity and myocardial depression, leading to systemic hypotension (Singh and Evans, 1997). In circulatory shock, there is massive release of NO, which leads to decreased systemic blood pressure and vasodilation (Änggård, 1994). Alterations in mean pulmonary artery pressure (MPAP) and/or pulmonary vascular resistance (PVR) can lead to changes in lung water balance through changes in hydrostatic fluid exchange across the lung membranes. There is experimental evidence that too little NO is available in patients with hypertension, angina and shock, and conversely, there is too much available in circulatory shock, stroke and inflammation (Änggård, 1994).

Shirai et al. (1996) used an x-ray television system to directly measure the interior diameter (ID) of small pulmonary arteries and veins (100 to 1100 μm ID) of cats in response to 5, 15 or 40 ppm NO inhalation. They also evaluated the effectiveness of inhalation of 40 ppm NO in reversing large anoxia-induced ID constrictions at various levels of the lung. The results showed that under normoxic conditions NO inhalation dilated both veins and arteries less than ~900 μm, while having little to no effect in larger vessels. The vasodilator response to inhaled NO was preserved under anoxic conditions.

Channick et al. (1994) evaluated the effect of inhaled NO in reversing hypoxic pulmonary vasoconstriction in dogs. The results showed that inhalation of both

40 and 80 ppm NO reduced hypoxia induced pulmonary hypertension, without significant MetHb formation. Young et al. (1999) evaluated the effects of 10 ppm inhaled NO in spontaneously breathing anaesthetized horses to determine whether inhalation of NO would selectively vasodilate ventilated areas of the lung. This would promote blood flow to the dilated areas and thereby reduce venous admixture by modulating intrapulmonary shunting. In contrast to the results of Channick et al. (1994), inhalation of 10 ppm NO had no significant effect on any cardiopulmonary function measured. There was a significant correlation between shunt fraction at 65 min after induction of anesthesia and body weight, but this relationship was not altered by NO inhalation. The authors speculated that this might have been due to compression atelectasis from the horses lying down for such a prolonged period of time. Although not mentioned by the authors, it is also possible that 10 ppm NO was insufficient to induce effects in horses.

Pulmonary vascular resistance is frequently elevated in congestive heart failure, although the mechanism of this secondary form of pulmonary hypertension is currently unknown. There have been reports of increased left ventricular end-diastolic pressure and episodes of pulmonary edema associated with inhaled NO in patients with preexisting left ventricular dsyfunction, raising concern that inhaled NO is a myocardial depressant (Argenziano et al., 1998). Argenziano et al. (1998) measured myocardial contractile state in piglets with heart failure. Inhalation of NO reduced mean pulmonary artery pressure and pulmonary vascular resistance. Although inhaled NO reduced pulmonary vascular resistance, there were no alterations in myocardial contractility or diastolic function. The results did not confirm case reports of myocardial depression associated with inhaled NO, suggesting that the observed myocardial depression may be related to increased volume delivery to the left side of the heart due to reduced pulmonary vascular resistance.

Pulmonary hypertension is defined as a mean pulmonary artery pressure > 20 mm Hg. Pulmonary hypertension can develop in normal lungs under conditions of alveolar hypoxia. Pathologic causes of pulmonary hypertension include parenchymal lung disease, pulmonary vascular obstruction (ie., thromboembolism), pulmonary venous hypertension (ie., left ventricular failure or mitral stenosis), intracardiac shunt (ie., septal defects in the atria or ventricles), drugs (ie., anorexic agents, cocaine), and idiopathic causes (primary pulmonary hypertension, or pulmonary hypertension of the newborn). The severity of pulmonary hypertension is at least partly determined by the relative balance between vasoconstrictor and vasodilator stimuli. Inhibition of NO by L-NMMA leads to a dose-dependent increase in pulmonary vascular resistance, indicating that basal vascular tone is determined, in part, by NO in both the pulmonary and systemic circuits. The most common cause of secondary pulmonary hypertension is hypoxia due to chronic obstructive disease (COPD) (Sperling and Creager, 1999).

The pulmonary vascular endothelium produces NO in fetal and newborn pulmonary vessels. The decline in pulmonary vascular resistance at birth is due to the increase in O_2 tension after birth, and is partly mediated by NO. In some infants this fails to occur, and the infant develops persistent pulmonary hypertension of the newborn (PPHN). There is some evidence that this condition is due to reduction in the bioavailability of NO, which leads to maintenance of a fetal circulatory pattern. The effect is likely due to a reduction in eNOS availability (Sperling and Creager, 1999).

Decreased NOS expression leads to reduced NO release. Expression of cNOS from endothelial cells (eNOS) in the lungs of normal subjects was greater than in the lungs of patients with primary and secondary pulmonary hypertension. There was little eNOS expression in severely abnormal arteries. Also, in patients with pulmonary hypertension, the less eNOS they produced, the more severe the arterial histological changes. This study also found abundant endothelin, a vasoconstrictor, in patients with pulmonary hypertension. These results point to the possibility that pulmonary hypertension may be due to an imbalance between vasodilation and vasoconstriction (Sperling and Creager, 1999).

Several mechanisms have been suggested, in addition to reduced eNOS availablility, to explain possible reduced bioavailability of NO. Insufficient L-arginine availability could also impair NO bioavailability, since L-arginine is the substrate for NO production by NOS, although there are questions about whether L-arginine can be rate-limiting, and whether such conditions actually occur. It is also possible that L-arginine is a limiting factor only in some forms of pulmonary hypertension. Although the ultimate effect is the same, it is possible that several different pathophysiologies may be responsible for different forms of pulmonary hypertension. Another possibility is that the severity of vessel wall dysfunction is an important factor (Sperling and Creager, 1999).

Zapol et al. (1994) studied the responses of anaesthetized, open-chested guinea pigs to methacholine bronchochallenge and NO inhalation. Inhalation of NO led to bronchodilation, beginning and ending quickly with application and withdrawal of NO. There was no enduring effect of NO once it was withdrawn, indicating that the effect was dependent on the continued presence of NO.

Emil et al. (1997) studied the relationship between inhaled NO dose and reduction in mean pulmonary artery pressure (MPAP) and pulmonary vascular resistance (PVR) during hypoxia in adolescent swine. The investigators manipulated the fraction of inspired oxygen to induce moderate or severe hypoxia. The pigs were maintained in the hypoxic state for 30 min., and were then treated with inhaled NO beginning at 5 ppm, and increasing doubling doses at 10 min intervals until the final NO concentration was 80 ppm. NO was effective in reducing MPAP in both moderate and severe hypoxia, although the slope of the dose-response relationship was steeper in the severely hypoxic group. Maximal dilation was achieved in the moderate hypoxia group with

inhalation of 5 to 10 ppm NO, while doses between 40 and 80 ppm were necessary in the severe hypoxia group. It is clear that NO acts according to a dose-response relationship, and that the degree of effectiveness is related to the degree of initial dysfunction.

Fredén et al. (1995) studied the effect of hypoxia, inhaled NO, and NOS inhibitors on pulmonary blood flow in pigs. The results indicate that hypoxia reduced pulmonary blood flow to hypoxic lung lobes. Pulmonary blood flow was further reduced in animals pre-treated with the NOS inhibitor L-NAME. Inhalation of 40 ppm NO increased lobar pulmonary blood flow under both hypoxic and L-NAME treatment conditions. Blood oxygenation was improved concomitantly with the reduction in pulmonary hypertension, and improved distribution of pulmonary blood flow to ventilated regions of the lungs. The findings suggest that one of the responses to NO inhalation is to improve ventilation-perfusion matching in the lungs, thereby improving blood oxygenation, and that inhaled NO can substitute for endogenous NO derived via the NOS pathway.

Inflammation and Immune Responses

Daher et al. (1997) reported that exposure to 20 ppm NO for two hours reduced the viability and superoxide anion production of neutrophils. The effect persisted, requiring over 4 days for restoration of neutrophil respiratory burst activity (Gessler et al., 1996). Bacha et al. (1997) reported that inhaled NO inhibited adhesion of neutrophils to pulmonary artery endothelial cells in a porcine model of transplantation reperfusion injury. NO has been reported to decrease viability of tumor cells (Hirano, 1997), and to increase production of IL-8 by neutrophils (Cuthburtson et al., 1997). Several investigators have described the inhibition of histamine release from basophils (Iikura et al., 1998) and mast cells (Masini et al., 1991 & 1994; Mannaioni et al., 1991) by nitric oxide. Since NO appears to be involved in inhibiting histamine release, the presence of NO synthase and NO may be important in limiting the extent of allergic responses. Finally, Hebstreit et al. (1998) found that NO specifically prevents apoptosis (programmed cell death) in freshly isolated human eosinophils. This may lead to accumulation of immune cells in the lungs of patients with allergies and/or asthma.

NO may participate in host defense against viral, bacterial, fungal and protozoal infections (Lundberg, 1996). Inflammatory cells, e.g., macrophages and granulocytes, express iNOS in response to a variety of infectious and inflammatory stimuli. iNOS induction mediates the “killer” function of these immune cells by showering invading cells with NO (an oxidizing agent). The molecular targets in the victim cells are Cu-Fe proteins, which after attack by NO, release Cu++ and Fe++, generate O_2 and highly toxic hydroxyl radicals. It should be noted that this chain of reactions could lead to pulmonary inflammation if sufficient quantities of these cytotoxic substances are released (Änggård, 1994). Increased iNOS expression has been associated with worsening of infectious disease, i.e., influenza, pneumonitis and such inflammatory states as

endotoxin-induced hypotension and autoimmune vasculitis (Steudal et al., 1999). In such cases increased NO release can lead to systemic vasodilation and the "low tone" state in sepsis (Steudel et al., 1999). In addition, there is evidence that endogenous NO suppresses plasma leakage in the airways, but that when iNOS is expressed, the increased production of NO enhances plasma leakage. These opposing findings suggest that NO may have a role in maintenance of airway function and in airway inflammatory diseases where iNOS is expressed (Bernareggi et al., 1997).

Exhaled NO is increased in patients with inflammatory lung conditions, including viral upper respiratory infections. Tsutsumi et al. (1999) reported that respiratory syncytial virus infection enhanced expression of iNOS in human type 2 alveolar epithelial cells. The same investigators also described a significant increase in iNOS gene expression in nasopharyngeal exudate cells obtained from infants during the acute phase of respiratory syncytial virus bronchiolitis. Infection with human rhinovirus results in production of IL-8, IL-6, RANTES, and GM-CSF in human respiratory epithelial cell lines (Tsutsumi et al., 1999). A NO donor (NONOate) inhibited rhinovirus replication and cytokine production (Sanders et al., 1998), suggesting that endogenous NO may play an anti-inflammatory and anti-viral role in colds. However, Murphy et al. (1998) reported that, based on the timing of exhaled NO changes relative to experimental influenza A infection, NO did not appear to directly contribute to illness manifestations. Other studies have reported that NO may have bacteriostatic effects (Hoehn et al., 1998), and that it may be involved in the epithelial damage seen in asthma, and infections with Pseudomonas and Pertussis (Dowling et al., 1998; Flak and Goldman, 1996). Loveless et al. (1997) reported that exhaled NO is decreased in patients who are HIV-positive, and that the decrease was not correlated with smoking or with CD4-positive T-cells numbers, suggesting that these patients may have a defect in one or more isoform of NOS.

The pathophysiological effects induced by group B streptococcus (GBS) are similar in newborn humans and animals, namely pulmonary hypertension, hypoxemia and V_A/Q mismatching. In piglets, GBS sepsis has two phases. The early phase, up to about one hour after induction of infection, is mediated by the arachidonic acid metabolite, thromboxane A_2, while the late phase, two to six hours after induction of infection, is associated with multiple vasoactive mediators and histologic evidence of vascular injury. NO inhalation is known to be effective in relaxing vascular smooth muscle when there is no pulmonary vascular injury. Since inhaled NO has been shown effective in reversing the adverse effects of neonatal pulmonary hypertension, Berger et al. (1993) studied the effect of inhaled NO on GBS sepsis in piglets to determine whether inhaled NO would also be efficacious in treating the late phase respiratory effects of GBS sepsis. The results showed that inhaled NO was equally effective in reversing pulmonary hypertension, hypoxemia and V_A/Q mismatching in both the early and late phases of GBS sepsis in piglets. This suggests that GBS sepsis does not

sufficiently injure neonatal pulmonary vascular smooth muscle that the response to inhaled NO is impaired.

Daher et al. (1997) investigated whether NO exposure altered the anti-microbial functions of neutrophils, including production of superoxide anion, O_2 consumption, and/or myeloperoxidase release with and without concurrent hyperoxia. Studies were performed with neutrophils isolated from whole blood. Neutrophils exposed to either 20% NO in room air, or 20% NO in oxygen showed suppressed superoxide anion production, compared to room air controls, indicating inhibition of neutrophil oxidative function. These observations may be at least partially due to an observed decrease in neutrophil viability. The resulting down regulation of neutrophil function, if significant enough, could result in reduced neutrophil effectiveness in combating infection.

Asthma and Airway Reactivity

NO appears to have multiple effects on asthmatic airways, in that it is both a mild bronchodilator, as well as a promoter of airway inflammation. NO mediates bronchodilation through its action as a neurotransmitter in the nonadrenergic noncholinergic system, although it is less potent as a bronchodilator than commonly used β-agonist medications. There is a measurable amount of NO in the exhaled air of humans. The exhaled NO concentration is higher in human subjects with inflammatory airway diseases, such as asthma, than in healthy subjects. Exhaled NO can be reduced in both healthy and asthmatic subjects by NOS inhibition, and in asthmatics by treatment with glucocorticosteroids (Curran, 1996).

Högman et al. (1993) measured specific airway conductance (SGaw) and functional residual capacity (FRC) in healthy nonsmoking adults, adults with hyperreactive airways, asthmatics and patients with chronic obstructive pulmonary disease (COPD) in response to inhalation of 80 ppm NO for 10 minutes. Inhalation of NO had no effect on baseline SGaw or FRC in normal subjects or those with hyperresponsive airways. The hyperreactive subjects were able to tolerate a higher inhaled dose of methacholine when they simultaneously inhaled NO. Asthmatics showed modest improvement in SGaw with NO inhalation, but no effect on FRC. Inhalation of NO and a β_2-agonist together induced greater bronchodilation than observed with either treatment alone. In contrast, COPD patients had no alteration in SGaw or FRC with NO exposure, although there was a small, but significant improvement in SGaw with the combination of inhaled NO and a β_2-agonist. In asthmatics, the effect of NO was small compared to that of the β_2-agonist, although the results suggest a synergistic effect between the two agents in both asthmatics and COPD patients.

Several researchers have observed that the NO concentration in exhaled air is increased in asthmatics, and have suggested that exhaled NO concentration might be a useful, noninvasive method for monitoring pulmonary inflammation.

Alving et al. (1993) reported that asthmatics exhaled more NO than healthy subjects, both nasally and orally. The results suggest that NO in exhaled air of normal subjects is mainly generated in the nasal mucosa, while asthmatics have increased pulmonary NO production. Atopic subjects exhaled 2-3 times as much NO with oral breathing as normal subjects. The source of endogenous NO is unknown, although the results suggest that monitoring of exhaled NO may be a useful, non-invasive method of tracking airway inflammation. Piacentini et al. (1999) studied 20 dust mite-sensitive asthmatic children (6-15 yrs.). When the children were removed from exposure to house dust mite allergen, exhaled NO decreased over several weeks. The investigators suggested that this might reflect a reduction in airway inflammation, although there were no direct measurements of airway inflammation.

Kanazawa et al. (1998) compared exhaled NO and the number of neutrophils in induced sputum samples in normal males, and male asthmatics and COPD patients. Exhaled NO concentration was higher in asthmatics than in normal subjects and COPD patients, who both had similar exhaled NO concentrations. Analysis of induced sputum showed that the neutrophil count was highest in the COPD patients, while the eosinophil count was highest in the asthmatic subjects. There were no differences in macrophages or lymphocytes among the three groups. Counts of both neutrophils and eosinophils were very low in the normal subjects. Asthmatics had a greater concentration of NO derivatives in induced sputum, compared to normal subjects and COPD patients, although the COPD patients had a higher concentration than normal subjects.

Takahashi et al. (1998) compared the effect of inhaled NO to that of a common β-agonist bronchodilator in healthy and asthmatic subjects who inhaled room air or air containing ppm NO for 10 min after completion of a methacholine bronchochallenge. NO concentrations of 5, 20 and 80 ppm were used with the healthy group, but only 80 ppm with the asthmatic group. The investigators did not measure any pulmonary functions. Inhalation of 80 ppm NO after methacholine-induced bronchospasm reduced SaO_2 and PaO_2. Evaluation of the dose-response data indicated that all concentrations of NO had a similar effect on SaO_2. Bronchodilator therapy often leads to reduction in SaO_2 and PaO_2 because of increased blood flow to poorly ventilated regions of the lungs, leading to reduction in V_A/Q matching. The worsening of bronchoconstriction-induced hypoxemia was similar to that observed with typically prescribed bronchodilators. Hypoxemia resulting from NO inhalation was improved when the O_2 concentration in the inhalation mix was increased.

Dupont et al. (1998) measured the effect of glucocorticoids on exhaled NO concentration in male and female asthmatics and normal subjects. The concentration of NO in exhaled air was inversely correlated with the histamine PC_{20} in asthmatics. Treatment with glucocorticoids reduced exhaled NO in asthmatics, suggesting a relationship between endogenous NO production and pulmonary inflammation. It should be noted that these were well-controlled mild

asthmatics. Whether these findings would be confirmed in less well-controlled or more severe asthmatics remains unresolved.

Blood

Chromosomal Changes

Luhr et al. (1998) evaluated chromosomal aberrations in peripheral blood leukocytes drawn from humans who inhaled 40 ppm NO for 2 hours. There was a statistically, though not clinically, significant increase in MetHb from 0.63 ±0.21% to 1.13 ±0.39% during NO inhalation. NO inhalation had no effect on hemoglobin (Hb), hematocrit, or leukocyte count, although there was a small, statistically significant decrease in platelet count. These investigators reported that inhalation of NO did not affect the frequency of chromosome aberrations in peripheral blood leukocytes, nor did it induce alterations in cell cycle kinetics. However, the potential for other types of chromosome changes was not evaluated in this study.

Platelet Function

There are conflicting data on the effect of NO inhalation on platelet function. Platelets have cNOS, but not iNOS, and thus generate NO constitutively. It has been hypothesized that constitutively produced NO may function as a negative feedback mechanism to inhibit platelet function. Albert et al. (1996) reported that inhalation of 30-80 ppm NO did not alter heart rate, blood pressure or O_2 saturation in arterial blood of healthy adult males. Under the conditions studied, NO inhalation had no consistent influence on platelet aggregation or secretion. In another study, published in 1999, Albert et al. (1999) investigated whether inhibition of NOS synthesis, and therefore endogenous NO production, would increase platelet activity, and conversely, whether inhalation of 30 ppm NO for 30 min would inhibit platelet function. The results showed no effect of NOS inhibition on platelets, in spite of clear cardiovascular effects. Neither treatment affected platelet aggregation. Similarly, Krejcy et al. (1995) reported that 16 healthy male subjects who inhaled 50 ppm NO for 30 min showed no effects on systemic blood pressure or heart rate, and a small, though not statistically or clinically significant, increase in MetHb. There were no significant effects on platelet function or aggregation, or on measures of hemostasis. In contrast, Gries et al. (2000) exposed 36 male and female subjects to 0 to 40 ppm NO by inhalation for 40 min followed by continued measurements during a 15 min room air recovery period. Platelet aggregation was inhibited with inhalation of all NO concentrations, while inhalation of 40 ppm NO led to a significant increase in bleeding time compared to room air inhalation. There was no difference between the responses of males and females. The discrepancies among these results may relate to experimental design or methods.

Acute pulmonary embolism is often accompanied by increased platelet aggregation, which complicates the clinical picture of increased mean pulmonary artery pressure. If circulating platelets are activated, the resulting aggregation can aggravate mechanical obstruction due to the embolism. Platelet aggregation can also be accompanied by release of various vasoactive substances, such as serotonin and thromboxane A_2 that induce vasoconstriction of the pulmonary vessels, thereby increasing pulmonary hypertension and hypoxemia. If severe enough, this chain of events can lead to circulatory failure. In such cases, prevention of platelet aggregation would be useful in reducing the harmful effects of pulmonary embolism.

Gries et al. (1997) studied the effects of inhaled NO on platelet aggregation after pulmonary embolism was induced in piglets by injection of microspheres. Once pulmonary hypertension was established, the piglets inhaled 5, 20, 40 or 80 ppm NO in a stepwise fashion, with each concentration maintained for 10 min. The results showed that NO inhalation after pulmonary embolism significantly decreased mean pulmonary artery pressure. NO inhalation inhibited the increase in platelet aggregation after acute pulmonary embolism. Because of the sequential exposure doses, and the short inhalation time at each dose, this study provides no evidence as to whether there is a dose-response relationship between inhaled NO and platelet aggregation, although NO inhalation did result in functional improvement.

Methemoglobin

NO binds with hemoglobin and forms methemoglobin (MetHb). A concern, particularly with clinical NO use, has been whether or not pathological increases in MetHb would occur, since NO binds to hemoglobin more strongly than O_2 does. Young et al. (1994) investigated the relationship between dose of inhaled NO, duration of inhalation, and MetHb formation in healthy human subjects who breathed 32, 64 or 128 ppm NO for three hours, or 512 ppm until MetHb concentration reached 5%. Blood was sampled throughout each exposure and for three hours afterward. It took about one hour with inhalation of 512 ppm NO inhalation for MetHb to reach 5%, at which time MetHb was still increasing. With the lower inhaled doses, MetHb concentration leveled off well below 5%. Evidence to date (Gladwin et al., 1999; Luhr et al., 1998; Young et al., 1994) suggests that a clinically significant increase in MetHb is not likely under typical clinical conditions due to the rapid biotransformation of the NO component of MetHb to nitrate and nitrite. It should be noted that these were acute exposures with healthy people. Patients and/or long-term exposure might have different results. Also, these results are not applicable to neonates, who have less capacity than adults to remove MetHb from their circulation.

Sickle-Cell Anemia

Sickle-cell anemia is a disease characterized by a single amino acid switch from valine to glutamic acid in the hemoglobin molecule. Sickle-cell hemoglobin (HbS) has a P50, which is higher than in individuals with normal hemoglobin. Since NO reduces hypoxemia in pulmonary hypertension, it has been hypothesized that NO inhalation might be efficacious in ameliorating hypoxemia in sickle-cell patients. Gladwin et al. (1999) had normal and sickle-cell anemia patients inhale 80 ppm NO for 2 hours. The sickle-cell patients had greater increases in MetHb, as well as plasma nitrate and nitrite concentrations with NO inhalation compared to the normal subjects. Inhaled NO did not improve baseline arterial or venous hypoxemia in the sickle-cell group, and had no effect on the oxygen dissociation curve, suggesting that inhaled NO is not useful as a potential treatment for sickle-cell disease.

Oxidant Injury

A key event in oxidant lung injury is migration and adherence of neutrophils to the pulmonary vasculature, and the local release of reactive oxygen-derived radicals from these neutrophils. NO can contribute to oxidant lung injury if its release is triggered during the inflammatory process (Steudel et al., 1999). Nelin et al. (1998) reported that NO inhalation improved the survival of rats exposed to a hyperoxic environment. Sprague-Dawley rats were exposed to: (1) >95% O_2, (2) >95% O_2 + 10 ppm NO, (3) >95% O_2 + 100 ppm NO, or (4) >95% O_2 + 3 ppm NO_2. After 120 hours of exposure, the >95% O_2 + 100 ppm NO group showed a much higher survival rate, 21/30, compared to 2/24 for >95% O_2 and 2/12 for the >95% O_2 + 10 ppm NO group. In contrast, survival at 120 hours of exposure to >95% O_2 + 3 ppm NO_2 was 1/12. Other rats were similarly exposed, but for 60 hrs, after which their lungs were assayed for total protein, reduced glutathione (GSH), oxidized glutathione (GSSG) and 4-hydroxy-2(E)-nonenal (4-HNE), and neutrophil accumulation. Although there are issues concerning the appropriate method of normalizing the biochemical data since the various exposure regimens altered the cell inventories of the lungs, the only factor which distinguished the rats exposed to atmospheres including NO from those not including NO was the amount of 4-HNE/mg protein. This supports the hypothesis that NO has an anti-lipid peroxidation effect in the lungs. Previous research has shown that oxidation of low density lipoproteins can be inhibited by NO (Garat et al., 1997; Hogg et al., 1993; Rubbo et al., 1994). These results suggest that ambient NO might provide protection from ambient oxidants.

Systemic Effects

There is some evidence from animal models that NO might also have systemic effects. Although the mechanism(s) is unclear, it is possibly related to reversible binding of NO to hemoglobin, and its subsequent release at distant sites (Steudel

et al., 1999). Fox-Robichaud et al. (1998) studied the effects of inhaled NO on vasoactivity, leukocyte-endothelial cell interactions, and endothelial dysfunction in the peripheral microvasculature of NO depleted vessels in a mesenteric venule of anaesthetized cats. The cats were ventilated with an air mixture containing 0, 20 or 80 ppm NO. The results suggested that inhalation of NO reduced adhesion of leukocytes, and vasoconstriction in the mesenteric blood vessels, as well as reducing vascular leakage. When the mesenteric tissue was depleted of endogenous NO, inhaled NO replaced it, and prevented the adverse effects of NO depletion. These results suggest that NO may be transported to non-pulmonary sites where it is physiologically active.

CLINICAL USE OF NO

There are several common drugs that reduce blood pressure by a NO mechanism that are used clinically, for example nitroprusside and nitroglycerine. Nitroprusside is more potent as an arterial vasodilator, while nitroglycerine is more potent as a venous dilator. Both are non-specific, in that they affect blood pressure throughout the body. This is a distinct drawback when the patient has pulmonary hypertension and normal systemic blood pressure. Since both pulmonary and systemic blood pressures are reduced by these drugs, the resulting reduced cardiac output will put the patient at greater risk than before treatment, in spite of the beneficial effect on the pulmonary circulation. The selectivity of inhaled NO to reduce pulmonary blood pressure makes it an attractive alternative to systemically active drugs. An additional benefit is that inhaled NO acts micro-selectively by dilating only the blood vessels adjacent to alveolar units that are ventilated. Areas with collapsed alveoli do not receive NO, and thus the blood vessels adjacent to them remain constricted. This largely limits improved ventilation and perfusion to the same areas, tending to improve V_A/Q matching, pulmonary blood flow and blood oxygenation (Gaston et al., 1994; Greene & Klinger, 1998; Lunn, 1995; Steudel et al., 1999). These features have led to evaluation of inhaled NO as a treatment for several clinical conditions.

Use of NO in Neonates

During the first few hours and days after birth, pulmonary artery pressure, pulmonary vascular resistance and impedance fall dramatically in normal infants, while pulmonary blood flow increases as a result of hemodynamic alterations resulting from birth and the necessary shift to air breathing. An infant's initial inhalations of ambient air, with its comparatively high O_2 content, lead to pulmonary vasodilation that appears to be NO-mediated. Evidence suggests that endogenous NO activity increases with advancing gestational age. Endogenous NO formation is present in the early stages of gestation, although ability to respond to stimuli that induce pulmonary vascular endothelial NO formation develops later, as does the vasodilatory response to high inspired O_2 concentration (Kinsella and Abman, 1997).

The pulmonary vascular, parenchymal and airway responses of the premature lung to mechanical ventilation are uniquely different from those of a term infant. Structural and functional pulmonary immaturity and surfactant deficiency characterize the lungs of premature infants, making them more prone to pulmonary hypertension, a major cause of morbidity in premature infants. There are several treatments currently used for neonatal pulmonary hypertension: drugs, hyperoxia and induced alkalosis. Unfortunately, each of the conventional treatments poses potential risks to the neonate. Drug treatment poses the risk of systemic hypotension, while hyperoxia poses a serious risk for oxygen-induced lung damage, and alkalosis presents the possibility of adverse effects on cerebral blood flow. A variety of permanent neurological deficits are common sequelae to reduced cerebral blood flow in neonates (Heidersbach et al., 1999; Kinsella & Abman, 1997; Kusuda et al., 1998; Lopes Cardozo et al., 1996).

Persistent pulmonary hypertension of the newborn (PPHN) is a clinical syndrome characterized by sustained pulmonary hypertension and severe hypoxemia, resulting in cyanosis unresponsive to O_2 therapy. Diagnostic confirmation of PPHN includes echocardiographic observation of right to left shunt through the ductus arteriosus or foramen ovale due to increased pulmonary vascular resistance (PVR) in the absence of congenital cardiac disease. NO inhalation therapy has been particularly successful is treating this condition. Since the activity of inhaled NO is limited to the lungs, there is no reduction in systemic blood pressure, as is the case with systemic nitrovasodilators such as nitroprusside. Inhalation of NO induces reduction in pulmonary capillary pressure, reduces the need for extra-corporeal membrane oxygenation, and although it does not necessarily affect survival, leads to improved V_A/Q matching (Kinsella & Abman, 1997).

Although NO inhaled has been used to treat neonatal pulmonary hypertension, and is known to reduce pulmonary vascular pressures, improve blood oxygenation, and improve VA/Q matching, little is known about the effects of NO inhalation on cerebral blood flow in human infants. Studies have, however, used neonatal animal models to evaluate the utility of NO as a clinical treatment for various lung problems in premature infants. Kusuda et al. (1999) measured cerebral blood flow in newborn piglets before and after pulmonary hypertension was induced by hypoxia. The piglets were each assigned to one of several treatment groups, including both conventional treatments and inhaled NO. The results showed that although inhalation of 30 ppm NO did not induce the largest reduction in pulmonary vascular pressures, it had no significant effect on systemic blood pressure, and did not alter cerebral blood flow as the various drug treatments did. This suggests that NO inhalation might reduce long-term effects of pulmonary hypertension treatment in neonates.

Studies of induced pulmonary hypertension in neonatal foals, piglets and lambs (Lester et al., 1999; Emil et al., 1995; Heidersbach et al. 1999) have shown that

inhalation of 5 to 160 ppm NO was effective in significantly reducing pulmonary hypertension, and in more moderate pulmonary hypertension, completely reversing it. There was a dose-response relationship between severity of pulmonary hypertension, degree of reversal, and NO concentration. In all three species, reduction of pulmonary arterial pressure led to improvement in arterial oxygenation and systemic O_2 delivery.

Myers et al. (1997) studied age-related differences in pulmonary artery response to hypoxia and inhaled nitric oxide in 48-hr and 14-day old piglets. The results indicated that inhibition of NO production potentiates hypoxic vasoconstriction at the level of the distal pulmonary arterioles in both groups of animals. However, the older group of piglets showed stiffening of the proximal pulmonary arteries that was not seen in the neonatal animals. This could explain why older infants do not always respond to inhaled NO in the same way as neonates, suggesting an age-related change in NO responsiveness.

Storme et al. (1998) examined whether inhaled NO altered oxidative stress parameters and lung inflammation in the lungs of prematurely born lambs as a model of hyaline membrane disease. Except for NO inhalation, treatment afforded the lambs was similar to that applied in veterinary clinical situations. The lambs were mechanically ventilated, with the experimental group ventilated with air containing 20 ppm NO, and the control group with room air for five hours. There were no differences between the two groups for any measured parameter of oxidative stress and lung inflammation. Thus, it does not appear that NO inhalation has a significant effect on oxidative stress or lung inflammation in a model of hyaline membrane disease.

Chronic Pulmonary Hypertension

Chronic pulmonary artery hypertension is characterized in early stages by partially reversible mean pulmonary artery pressure (MPAP). Cases of advanced disease are characterized by non-reactive, irreversible remodeling of the pulmonary vasculature. NO inhalation is used as a test to determine the stage of the disease. If NO inhalation reduces MPAP, the disease is still reversible, and NO is one of the available treatment modalities (Steudel et al., 1999).

Acute (Adult) Respiratory Distress Syndrome (ARDS)

This pathophysiological condition results from a diffuse set of pulmonary insults. It is characterized by acute pulmonary arterial hypertension due to pulmonary vasoconstriction and occlusion of the pulmonary vasculature, along with intrapulmonary shunting secondary to a loss of hypoxic pulmonary vasoconstriction, resulting in hypoxemia. Pulmonary arterial hypertension, in concert with altered vascular permeability contributes to pulmonary edema, which further hinders gas exchange. This combination of events can lead to an

increase in right ventricular afterload, and ultimately to right ventricular dysfunction (Cioffi & Ogura, 1995).

Factors thought to contribute to pulmonary hypertension in ARDS include circulating cytokines, obliteration of the vascular bed, and impairment of cGMP production, with the degree of pulmonary hypertension correlating with the severity of lung injury. Conventional treatment of ARDS with systemic vasodilators such as nitroprusside or nitroglycerine, lowers arterial pressure in both the pulmonary and systemic vascular circuits. Use of these vasodilators can lead to systemic hypotension and cardiac ischemia, because they act non-selectively in the pulmonary circuit, leading to dilation in both well and poorly ventilated alveolar units, leading to a reduction in oxygenation (Greene & Klinger, 1998).

The advantages of inhaled NO therapy include that it is active only in the pulmonary circulation, having no systemic effect. The vasodilatory effect is limited to ventilated areas of the lung, so V_A/Q matching is improved, along with arterial oxygenation (Sperling and Creager, 1999), although the effects persist only as long as NO continues to be inhaled.

Cioffi and Ogura (1999) reported that NO inhalation in ARDS led to a small mean reduction in pulmonary artery pressure, and an increase in oxygenation, but the changes were small and clinically insignificant. The patients varied widely in their response to inhaled NO, some having large improvements, and some having none. This may be due to the fact that although all of the patients had pulmonary hypertension, they were diverse in terms of the underlying cause of their disease. The different etiologies of their disease may account for the differences in responses. The utility of inhaled NO in reducing pulmonary hypertension appeared to be greatest in clinical conditions where reactive pulmonary arterial hypertension was the predominant feature. It did not seem efficacious when there was extensive pulmonary inflammation, or when increased blood flow was directed to poorly ventilated lung segments. Further, observed improvements in oxygenation and reduction in pulmonary artery pressure persisted only while the patient continued to inhale NO.

Girard et al. (1992) evaluated the effects of NO inhalation on pulmonary hemodynamics in patients with pulmonary arterial hypertension after mitral valve replacement. The patients inhaled 40 ppm NO for 10 min during the first 24 hours post-mitral valve replacement surgery. There were small, though clinically beneficial, changes in hemodynamics and oxygenation, although they disappeared as soon as NO was withdrawn.

Frostell et al. (1993) induced pulmonary hypertension and hypoxemia in healthy subjects by having them inhale a gas mixture containing 12% O_2 as a model of ARDS. The subjects then inhaled 40 ppm NO for 10 min during hypoxia. Inhalation of NO led to reduction in pulmonary arterial pressure, and improved

oxygenation, although the effect continued only as long as the subjects inhaled NO. The results suggested that vasodilation was restricted to the pulmonary circulation, as there were no changes in systemic pressures. These investigators also investigated the time course of the response to inhaled NO. Pulmonary artery pressure declined within one minute of beginning NO inhalation, and reversed as rapidly once NO inhalation ended.

Research on animals provides confirmation of the action of NO in models of ARDS. Several studies have shown that inhalation of 5 to 80 ppm NO reduces or completely reverses the hypoxic vasoconstrictor response, as well as pulmonary hypertension caused by hypoxia and thromboxane analogues in lambs (Kobayshi et al., 1996), and a porcine (Fredén et al., 1995) model of ARDS. Prostaglandin $F_{2\alpha}$ is a vasoconstrictor, and has been reported to enhance pulmonary vasoconstriction in hypoxic regions of the lungs, thereby redistributing blood flow away from poorly oxygenated regions of the lungs. Kobayashi et al. (1996) hypothesized that treatment with a combination of NO inhalation, which also redistributes pulmonary blood flow to well ventilated lung areas, and intravenous prostaglandin-F-2α ($PGF_{2\alpha}$) would have beneficial effects on pulmonary circulation, cardiac output, and blood oxygenation in an ovine model of adult respiratory distress syndrome. Treatment with $PGF_{2\alpha}$ alone improved arterial oxygenation due to a decrease in cardiac output, which was not altered by NO inhalation. Functionally, it appears that NO acted in the vicinity of the alveoli, while $PGF_{2\alpha}$ seemed act in the upstream resistance vessels in this model of ARDS.

NO is valuable in treating ARDS in that it selectively dilates only vessels in aerated alveoli, improving V_A/Q matching, and therefore oxygenation. The rationale for using NO with ARDS is primarily that if improvement in oxygenation is sufficient, the fraction of inspired O_2 and pulmonary ventilation pressure can be reduced, thereby reducing O_2 toxicity and barotrauma to the lungs. There is some divergence in outcomes with the use of NO for treatment of ARDS in terms of hemodynamic and respiratory effects. These finding suggest that pre-existing disease, other concurrent therapies, and possibly the duration and/or severity of pulmonary failure are factors in the efficacy of inhaled NO therapy in ARDS (Cioffi & Ogura, 1999). As with children, NO inhalation may not alter survival, although the need for more intensive or invasive treatments tends to be rendered unnecessary (Greene & Klinger, 1998).

NON-RESPONDERS

There are patients who do not respond to NO inhalation, the number reported as being between 30-45% (Steudel et al., 1999). Some hypotheses as to why include the clinical status of the patient, and the patient's clinical diagnosis. Hypo-responsiveness may be associated with decreased pulmonary cGMP release, suggesting down-regulation of the NO production system. Non-responsiveness has also been associated with increased endogenous production

of superoxide anion. One study has suggested a linkage to the ABO blood groups, with individuals with ARDS having blood types B and AB showing less response to inhaled NO than those with A and O blood groups (Weimann et al., 1998).

POSSIBLE SIDE EFFECTS OF NO INHALATION

There have been few reports of clinical NO toxicity, although these were at NO concentrations considerably above ambient, as well as above typical clinical concentrations (Greene & Klinger, 1998; Steudel et al., 1999). Although there is little information indicating harmful effects of inhalation of up to 80-100 ppm NO for time periods ranging from a few minutes to days, several potential side effects have been suggested. Inhalation of NO may vasodilate the pulmonary circulation, thereby increasing blood flow to the left ventricle. This in turn could increase left atrial pressure and pulmonary capillary wedge pressure (PCWP). This combination of events can lead to pulmonary edema, particularly in patients with markedly impaired left ventricular function (Greene & Klinger, 1998).

It is important to minimize NO contact with oxygen since NO readily converts to NO_2 in the presence of oxygen. NO_2 is a more potent toxic agent than NO, and provides none of the benefits described for NO (Lunn, 1995).

There is concern that the powerful cytotoxic properties of NO, which under some conditions are beneficial, could be detrimental if directed toward the host itself. Excess release of iNOS could lead to a role for NO in inducing inflammation (Lundberg, 1996). There is evidence that asthmatics and individuals with various lung infections exhale more endogenously produced NO than healthy individuals (Alving et al., 1993; Massaro et al., 1995). This suggests that NO may be a factor in induction of the inflammatory process and its perpetuation. If this is true, it is theoretically possible that inhaled NO could exacerbate inflammation in asthmatics and individuals with lung inflammation due to other causes. A potential issue in this regard is that it is not entirely clear how well exhaled NO correlates with airway inflammation, especially in conditions other than asthma. It has been suggested that exhaled NO could be a non-invasive method of measuring lung inflammation (Dupont et al., 1998; Kanazawa et al., 1998; Kharitonov et al., 1995; Lundberg, 1996).

There has been a suggestion of rebound pulmonary arterial hypertension with increased right to left shunting, along with decreased arterial blood oxygenation on discontinuation of inhaled NO therapy (Lunn, 1995). This accords with the fact that effects of inhaled NO seem to persist only while NO in being inhaled.

INTERACTIONS BETWEEN ENDOGENOUS AND AMBIENT NO

Interaction between ambient NO and endogenously produced NO is potentially important. Since endogenous NO is involved in many regulatory, inflammatory and immune functions, and appears to play a key role in defense against infection, it is conceivable that low concentrations of ambient NO which in themselves have no direct toxic effect, might down-regulate endogenous NO production, thereby altering baseline physiology and host defense.

There is some evidence to support this hypothesis. Several cell culture studies have demonstrated that NO exerts an inhibitory feedback effect on NOS (Buga et al., 1993; Assreuy et al., 1993; Rengasamy & Johns, 1994). Cigarette smoke contains a high concentration of NO, and the concentration of exhaled NO is significantly lower in smokers than in nonsmokers (Hill et al., 1995; Kharitonov et al., 1995; Persson et al., 1994). Furthermore, smoking a single cigarette significantly decreased exhaled NO (Kharitonov et al., 1995).

Piacentini et al. (1998) measured the exhaled NO concentration in two groups of subjects in a study designed to investigate whether environmental concentrations of NO in the range of 0 to 150 ppb influenced the exhaled NO concentration. Group 1 was tested when the environmental NO concentrations were 0 to 3 ppb and 20 to 60 ppb. Group 2 was studied when the ambient NO concentrations were 0 to 3 ppm, 80 to 100 ppm, and 120 to 150 ppm. The results suggest that ambient NO concentrations up to 150 ppb had no effect on exhaled NO concentration. In a similar study, Borland et al. (1993) reported that 0 to 7 ppb environmental NO had no effect on exhaled NO. However, both of these studies examined the effect of environmental NO on <u>baseline</u> exhaled NO. Neither examined exhaled NO as a response to a stimulus, e.g., respiratory infection, allergen exposure or asthma.

Van Amsterdam et al. (1999) measured exhaled NO once daily on 14 consecutive days in 16 healthy non-smoking adults in a study focused on whether ambient air pollution exposure had an effect on endogenous NO production. The subjects were exposed to varying concentrations of outdoor and indoor air pollutants during the period of the study. The subjects breathed NO-free air for one minute prior to providing an exhaled air sample for endogenous NO measurement to avoid sample contamination by ambient air. Exhaled NO was increased on days with elevated ambient NO and CO, although the effect did not carry over from previous days. The authors believed that CO served as a surrogate for some other ambient pollutant. The authors further speculated that increased exhaled NO was associated with ambient air pollution induced airway inflammation, although there were no direct measurements made that could confirm or refute the hypothesis.

The same research group performed a related study on both smokers and nonsmokers. Steerenberg et al (1999) found that endogenous NO in exhaled air was increased on days with high outdoor pollution. The association between exhaled NO was strongest with the ambient NO concentration. Studies with 4 smokers, each smoking one cigarette, showed elevated NO in exhaled air during smoking, most likely related to the high concentration of NO in the inhaled smoke. When the subjects performed a wash-out procedure and then provided an exhaled air sample that would be representative of endogenous NO production, there was no difference with the results of nonsmokers. Although the authors attributed the results to ambient NO, the validity of the methods, small study group and interindividual variability suggest that more investigation must be undertaken in this area before conclusions can be reached.

CONCLUSIONS

The explosion of information about the ubiquity and wide-ranging activity of NO in the body clearly suggests that early studies that concluded NO inhalation had no significant effects on human health did not examine the correct endpoints. While it is clear that NO is biologically active throughout the body, and that ambient NO could, at least theoretically, affect endogenous NO production and/or induce health effects, there is insufficient evidence available at this time to warrant consideration of an ambient air quality standard for NO. There is little basic biological or clinical literature available that directly addresses issues critical to assessment of possible health effects from exposure to ambient NO. The literature reviewed above indicates that NO can be both beneficial and injurious, and suggests that even the comparatively low concentrations of NO in the ambient air could affect cardiopulmonary regulation, pulmonary inflammation, asthma, and other inflammatory lung diseases. There is also evidence suggestive of possible effects on host defense, cell-mediated immune responses and platelet function.

RESEARCH RECOMMENDATIONS

The literature reviewed above suggests several avenues for future research relevant to air quality health effects and regulation. The answers to these questions, while not addressing all of those raised by the NO literature, would be particularly fundamental in determining whether or not an ambient air quality standard for NO should be considered.

(1) Does ambient NO exposure lead to biochemical and/or cellular changes in the lungs (cytokine release, or migration of inflammatory cells to the lungs) indicative of pulmonary inflammation?

(2) Does environmental NO exacerbate or ameliorate asthma or other inflammatory lung diseases?

(3) Is there a benefit from environmental NO? For example, does ambient NO inhalation contribute to host defense? Does its bronchodilator activity counteract O_3-induced bronchoconstriction? Does it reduce pulmonary inflammation due to oxidant air pollutant exposure?

(4) Does ambient NO alter platelet function?

(5) Does inhaling ambient concentrations of NO alter endogenous NO production, ie., does ambient NO inhalation down-regulate endogenous NO production?

(6) Does inhalation of ambient NO lead to increased V_A/Q mismatching in persons already suffering from V_A/Q mismatch-induced hypoxemia?

(7) Could NO be responsible for the mortality/morbidity findings in recent epidemiological investigations that have been attributed to NO_2, CO and/or PM?

Table 1: Nitric Oxide References

Clinical Use/ Neonates

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
Newborn lambs n=6 4-9 days	Cerebral blood flow, cerebral activity, blood gases in pulmonary circulation, effluent blood pressure in pulmonary artery and aorta	2-60 ppm stepwise	5-10 min at each dose	NO inhalation decreases pulmonary artery pressure with no effect on cerebral blood flow, electrocortical activity or blood gases. Cerebral blood flow was indirectly influenced by NO mediated decrease in CO_2 pressure.	Lopes Cardozo, et al, 1996
Newborn lambs n=14 1-6 days	Vascular pressure, left pulmonary artery blood flow	0, 5, 40, 80 ppm @ F_iO_2 = 0.21, 0.50, 1.0 0, 5, 40, 80 ppm @ arterial pH 7.3, 7.4, 7.5, 7.6	10 min steady state at each combination	Alkalosis. NO had greatest effect in decreasing pulmonary hypertension. F_iO_2 > 0.5 was not beneficial.	Heidersbach, et al, 1999
Premature lambs n=8 (experimental) n=10 (control)	Biochemical indices of oxidative stress. Inflammatory cells in bronchoalveolar lavage fluid influence cells in lung parenchyma.	20 ppm 0 ppm	5 hrs 5 hrs	No significant changes in oxidative stress parameters. No significant changes in number of inflammatory cells in bronchoalveolar lavage fluid or lung parenchyma.	Storme, et al, 1998
Pigs n=25	Platelet aggregation following induced pulmonary embolism	5, 20, 40, 80 ppm stepwise	10min / [NO]	NO inhibited platelet aggregation, decreased mean pulmonary artery pressure, increased end tidal CO_2, and increased mean systemic blood pressure.	Gries, et al, 1997

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
Yorkshire pigs n=7 12-16 wks	Pulmonary hypertension, cardiac output, hemodynamic parameters, arterial blood gases	5, 10, 20, 40, 80 ppm stepwise	10min / [NO]	Influence of NO inhalation on different degrees of hypoxia-induced pulmonary hypertension. Severe hypoxia predicted weaker improvement in pulmonary hypertension during NO therapy and stronger recurrence after discontinuing NO inhalation. NO also appeared to play a hemodynamic supportive role during severe hypoxia by enhancing cardiac output and preventing acute cor pulmonale. Lower inhaled doses of NO were less effective in reversing severe pulmonary hypertension than moderate pulmonary hypertension. The degree of hypoxia influences the effectiveness of an inhaled NO concentration, i.e., the more severe the hypoxia, the higher the NO concentration required for vasodilatation.	Emil, et al., 1995 Emil, et al., 1997
Yorkshire pigs 48 hr and 14 days n=8 per age group	Pulmonary arterial response to hypoxia Endogenous NO production was pharmacologically blocked	100 ppm		Model designed to represent pulmonary hypertension due to endothelial dysfunction in neonates and older infants. The data indicate that inhibited NO production potentiates hypoxic pulmonary vasoconstriction in the distal pulmonary arterioles of both age groups of animals. In contrast, only the older animals had increased vasoconstriction in larger, more proximal vessels with hypoxia. Inhaled NO effectively reduced arteriolar vasoconstriction.	Myers, et al., 1997
Foals n=5 1 to 3 days old	Systemic and pulmonary arterial pressure, arterial oxygen saturation	20, 40, 80, 160 ppm	6 min/concentration	Inhalation of 80 ppm NO at baseline had no effect on any measurements. U46619 induced pulmonary hypertension was reversed by inhalation of all NO concentrations. After pulmonary arterial pressure had returned to normal, hypoxia was used to induce pulmonary hypertension. Inhalation of 80 ppm NO reduced pulmonary artery pressure to a level below that present before hypoxia was induced.	Lester et al., 1999
Newborn piglets 1-3 days old n=15	Mean pulmonary arterial pressure, mean systemic arterial pressure, cerebral blood flow volume	30 ppm	N/A	Standard drug treatments for pulmonary hypertension led to reduced cerebral bloodflow, a risk factor for development of neurological deficits. Inhaled NO reduced pulmonary hypertension without alteration of cerebral blood flow.	Kusuda et al., 1999

Chromosomal changes					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
n=10 male and female	Chromosome aberrations measured in cultured blood lymphocytes obtained from NO exposed human subjects	40 ppm	2 hrs	No detectable changes in chromosomal aberrations in cultured in blood lymphocytes.	Luhr, et al, 1998

Platelets					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
Healthy males n=19 18-41 years	Platelet aggregation, bleeding time, platelet secretion products, cGMP	30, 80 ppm 30 ppm	15 min 55 min	No effects on heart rate, blood pressure, S_aO_2. Plasma cGMP increases with NO inhalation. Platelet aggregation and secretion not affected. Bleeding time significantly increased with 55 min at 30 ppm exposures.	Albert, et al, 1996
Healthy males n=14 18-25 years	Platelet aggregation, bleeding time, cGMP secretion production following inhibition of endogenous NO production	30 ppm	30 min	Circulating platelets were not influenced by depletion of endogenous or inhaled NO.	Albert et al., 1999
Healthy males n=16 nonsmokers 22-33 years	Half of subjects inhaled NO, the other half received an NO synthase inhibitor Measured biochemical indices of platelet activation, coagulation activation, platelet prostaglandin synthesis	50ppm	30 min	Neither inhalation of NO nor inhibition of NO synthesis had a major impact on hemostatic system activation in vivo in healthy humans.	Krejcy et al., 1995
Pigs with massive acute pulmonary embolism	Platelet aggregation	5, 20, 40, 80 ppm	Serial exposure for 10 min per dose	NO inhalation led to a significant decrease in initial and maximal platelet aggregation. Inhaled NO has a systemic and rapidly reversible inhibitory effect on platelet aggregation after acute pulmonary embolism in pigs.	Gries et al., 1997

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
Healthy males and females n=36	Platelet aggregation, bleeding	0, 1, 3, 5, 10, 40 ppm	40 min	NO inhalation inhibited platelet function, but the effect was not dose dependent. It was significantly decreased with inhalation of 5 and 10 ppm NO, but did not increase with administration of higher NO concentrations.	Gries et al., 2000
Clinical Use/Acute (Adult) Respiratory Distress Syndrome					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
Humans with mitral valve replacement n=6	heart rate, blood pressures, pulmonary capillary wedge pressure, central venous pressure, cardiac output, cardiac index, systolic index, systemic vascular resistances, pulmonary vascular resistance, left and right ventricular systolic work indices, arteriovenous O2 content difference, O2 delivery, O2 consumption and O2 extraction	36.8-38.4 ppm	10 min during NO inhalation, and 30 min after NO was discontinued	NO inhalation resulted in selective vasodilatation of the pulmonary vasculature, resulting in a reduction in pulmonary arterial pressure, but no change in systemic pressures. Methemoglobin formation was insignificant.	Girard et al., 1992
Suffolk lambs n=13	Hemodynamics and gas exchange	60 ppm	Measurements after 15 min PGF2a infusion; and after 30 min NO inhalation	Inhalation reduced critical vascular pressure near the alveoli without affecting the critical pressure, while PGF2a infusion constricted larger upstream pulmonary arteries, without affecting critical pressure. Addition of PGF2a to redistribute blood flow did not enhance the effect of NO.	Kobayashi et al., 1996
Swedish country-bred pigs n=21	Mean arterial pressure, mean pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, blood gases	40 ppm	30 min	Hypoxia-induced pulmonary vasoconstriction was decreased by inhalation of NO.	Fredén et al., 1995

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
Healthy adults n=9	Pulmonary and radial artery pressures	40 ppm	10 min	Inhalation of NO selectively induced pulmonary vasodilatation and reversed hypoxic pulmonary vasoconstriction without causing systemic vasodilatation in humans with hypoxia-induced pulmonary hypertension.	Frostell et al., 1993
Cardiopulmonary System					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
Cats n=7	Pulmonary artery and vein internal diameters	5, 15, 40 ppm	N/A	Investigated change in ID of pulmonary arteries and veins with ID of 100 to 1000 µm. Under normoxic conditions 5-40 ppm NO inhalation significantly increased the ID of arteries and veins smaller than ~900 µm in a dose dependent fashion. During anoxia, NO at these concentrations of NO also effectively reversed vasoconstriction.	Shirai et al., 1996
Dogs n=6	Acute hypoxic pulmonary vasoconstriction	40, 80 ppm	30 min	Inhalation of 40 and 80 ppm NO for 30 min rapidly reversed hypoxic pulmonary vasoconstriction in all animals, with no significant formation of NO_2 or methemoglobin.	Channick et al., 1994
Horses n=7	Venous admixture, pulmonary hemodynamics during halothane anesthesia.	10 ppm	20 min	No significant effect on any measure of cardiopulmonary function. Significant correlation between shunt fraction at 65 min from anesthesia induction and body weight, but this was not altered by NO inhalation. Authors speculated that this might have been due to compression atelectasis from the horses lying down for so long.	Young et al., 1999
Yorkshire pigs (F) n=10	Myocardial contractility in a model of ventricular failure and pulmonary hypertension	20, 40 ppm	N/A	Inhalation of NO reduced pulmonary vascular resistance, but did not alter myocardial contractility or diastolic function. The results suggest that increases observed in left ventricular end-diastolic pressure during inhaled NO therapy are not due to myocardial depression, but may be due to increased volume delivery to the left heart due to reduced pulmonary vascular resistance.	Argenziano et al., 1998
Guinea pigs	Bronchodilation in animals with methacholine-induced bronchoconstriction	5-300 ppm	N/A	Inhalation of NO produced rapid, dose-dependent reduction in R_L and increased C_{dyn}. The bronchodilator effects of NO were produced locally, and there was no systemic vasodilation or hypotension due to inhaling NO.	Zapol et al., 1994

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
Swedish country-bred pigs n=21	Distribution of pulmonary blood flow during regional hypoxia with and without IV nitric oxide synthase inhibition	5, 10, 20, 40, 80, 160 ppm	30 min	NO inhalation improved ventilation-perfusion matching in the lugs, thereby improving blood oxygenation. Inhaled NO could substitute for endogenous NO derived via the NOS pathway.	Fredén et al., 1995
Yorkshire pigs n=7	Effectiveness of NO in reducing pulmonary hypertension in relation to severity of hypoxia	5, 10, 20, 40, 80 ppm	10 min per dose	Degree of hypoxia affected response to inhaled NO. Lower NO doses were significantly less effective in achieving maximal pulmonary vasodilation during severe hypoxia.	Emil et al., 1997
Yorkshire pigs n=7	Influence of severity of hypoxia on response to NO in a model of pulmonary hypertension	5, 10, 20, 40, 80 ppm	10 min per dose	Severe hypoxia was predictive of lesser improvement in pulmonary hypertension with NO inhalation, and stronger recurrence with NO withdrawal. NO inhalation improved cardiac output during severe hypoxia.	Emil et al., 1995
Inflammation and Immune Responses					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
Human adults with HIV infection n=36	Exhaled NO			HIV patients exhaled less NO than healthy subjects. Since NO is a means of host defense against bacterial, viral and fungal infections, reduced exhaled NO may indicate a mechanism for impaired host defense in HIV infection.	Loveless et al., 1997
Human neutrophils	Oxidative function of neutrophils *in vitro*	5, 20 ppm		Exogenous NO exposure decreased neutrophil oxidative function, primarily as a result of reduced cell viability.	Daher et al., 1997
Human infants with pulmonary hypertension n=15	Superoxide anion production of neutrophils	N/A		Inhalation on NO reduced superoxide anion production by neutrophils stimulated by E. coli beginning about 24 hr from initiation of NO inhalation therapy. The effect was more pronounced after 72 hr of therapy, and continued for more than 4 days after the end of NO therapy.	Gessler et al., 1996
Pigs n=36	Effect of NO inhibition of neutrophil activation on ischemia-reperfusion injury with lung transplantation	30 ppm		Inhaled NO attenuated ischemia-reperfusion injury after non-heartbeating-donor lung transplantation, likely due to prevention of pulmonary vasoconstriction and to a direct effect on peripheral blood neutrophil adhesion to the endothelium, resulting in reduced neutrophil sequestration and tissue injury.	Bacha et al., 1997

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
Lewis lung carcinoma and B16 melanoma-4A5 tumor cells, injected into mice	Cytotoxic effects of NO on metastatic cell lines.	10-80 ppm	14 or 21 days	Inhaled NO did not reduce tumor colony formation in the lungs of mice.	Hirano, 1997
Human type 2 alveolar epithelial cells	Molecular mechanisms whereby respiratory syncytial virus affects endogenous inducible nitric oxide synthase production			Inflammatory cytokines, along with respiratory syncytial virus enhanced iNOS and endogenous NO production. This could explain why respiratory syncytial virus infection appears to aggravate pulmonary congestion in patients with congenital heart disease with left to right shunts.	Tsutsumi et al., 1999
Human trachael epithelial cells	Kinetics and mechanisms of interleukin-8 and interleukin-6 production from rhinovirus infected epithelial cells			Endogenous NO production inhibited rhinovirus replication and cytokine production in a dose-dependent fashion, without reducing levels of cytokine mRNA.	Sanders et al., 1998
Human basophils Rat peritoneal mast cells	Anti-IgE and ionophore A23187-induced histamine release			Exogenous NO played a role in regulation of basophil and mast cell activation.	Iikura et al., 1997
Rat mast cells	Synthesis of NO-like factor by mast cells, and its interaction with histamine release			Mast cell histamine release can be modulated by intrinsically generated NO.	Masini et al., 1991
Rat serosal mast cells	NO and superoxide regulation of histamine release by mast cells			Regulation of mast cell histamine release is partly through a NO-mediated mechanism.	Mannaioni et al., 1991
Bacterial cultures	Antimicrobial properties of NO at concentrations used to treat pulmonary hypertension in neonates	40, 80, 120 ppm	24 hr	NO has selective bacteriostatic effects on some common bacteria. The effect was dose-dependent, and occurred in the upper range of dosages used with NO inhalation therapy, however, a bacteriostatic effect would not be expected in the range of NO concentrations used with neonates and premature infants (1-80 ppm).	Hoehn et al., 1998

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
Human nasal tissue cultures infected with *Pseudomonas aeruginosa*	Effect of nitric oxide synthase inhibitor on *Pseudomonas aeruginosa* infection.			*Pseudomonas aeruginosa* stimulates inducible nitric oxide synthase expression, and the resulting NO may be a mediator of epithelial damage caused by *Pseudomonas aeruginosa.*	Dowling et al., 1998
Human eosiniphils in culture	Role of NO in preventing Fas receptor-mediated apoptosis			NO concentrations within allergic inflammatory sites may be important in preventing Fas receptor mediated apoptosis of eosinophils.	Hebestreit et al., 1998
Rat serosal mast cells	Effect of nitrovasodilators on mast cell histamine release			Nitrovasodilators inhibit release of mast cell histamine through generation of nitric oxide.	Masini et al., 1994
Human polymorphonuclear leukocytes	Effect of a nitric oxide donor and a combined nitric oxide-superoxide donor on IL-8 accumulation in cultured human neutrophils			Incubation of cells with an NO donor led to increased IL-8 production, while concurrent NO-superoxide donor led to decreased IL-8 production.	Cuthbertson et al., 1997
Piglets 14 days old n=10	Effect of NO inhalation on Group B streptococcal (GBS)induced pulmonary hypertension and ventilation/perfusion mismatch during early and late phase GBS-induced pulmonary hypertension	150 ppm	30 min	NO inhalation selectively reversed early- and late-phase GBS-induced pulmonary hypertension, with NO equally effective in both phases. Inhaled NO did not reverse GBS-induced ventilation/perfusion mismatching during the late-phase of sepsis.	Berger et al., 1993
Human polymorpho-nuclear leukocytes (PMNs)	Interleukin-8 (IL-8) production by PMNs with and without lipopolysaccharide and in the presence of either a nitric oxide donor or a combined nitric oxide and superoxide donor		20 hours	Combined nitric oxide-superoxide donor treatment dose-dependently decreased lipopolysaccharide-mediated IL-8 accumulation. Nitric oxide donor alone increased IL-8 accumulation.	Cuthbertson et al., 1997

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
Healthy adults with no history of asthma, allergy or sinusitis n=14	Exhaled NO during experimentally induced influenza A infection			Experimental influenza virus infection can increase oral by not nasal exhaled NO concentrations. The timing of exhaled NO changes over the course of influenza infection suggests that NO does not contribute to illness manifestations directly.	Murphy et al., 1998
Wistar rats males	Lipopolysaccharide-induced microvascular plasma leakage			Under baseline conditions, endogenous NO appears to suppress plasma leakage, but iNOS is expressed, the enhanced NO production enhances plasma leakage.	Bernareggi et al., 1997
Freshly isolated human eosinophils	Role of NO in apoptosis of eosinophils			NO prevented Fas receptor-mediated apoptosis in freshly isolated human eosinophils, possibly through preventing activation of a protease that targets lamin B_1. The results suggest a role for NO in the eosinophil apoptotic signaling pathway, and therefore, NO at allergic inflammatory sites may be a factor in determining whether an eosinophil survives or undergoes apoptosis upon Fas ligand stimulation.	Hebestreit et al., 1998

Asthma and Airway Reactivity

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
Healthy adults, adults with hyperreactive airways, asthmatics, patients with COPD	SGaw	80 ppb	10 min	No effect in healthy subjects or those with COPD. Modulated response to methacholine provocation toward bronchodilation. Weak bronchodilatory effect in asthmatics, but not in COPD patients.	Högman et al., 1993
Healthy and asthmatic adults	Exhaled NO			In control subjects, majority of exhaled NO appeared to originate in the nasal airways. In mild asthmatics, exhaled NO primarily came from the lower airways, and was 2-3 times higher than healthy subjects.	Alving et al., 1993
Allergic, asthmatic children	Exhaled NO as a marker of allergic inflammation			Allergen avoidance was associated with a decrease in exhaled NO, suggesting a reduction in allergic airways inflammation.	Piacentini et al., 1999

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
Children with mild asthma, healthy adults	Influence of ambient NO concentration on exhaled NO concentration			Ambient NO concentration had no effect on level of exhaled NO.	Piacentini et al., 1998
Healthy adults, asthmatics, COPD patients	Endogenous NO production			Asthmatics and COPD patients had greater NO production than healthy subjects. NO derivatives in induced sputum provided better assessment of airway inflammation in COPD patients than exhaled NO.	Kanazawa et al., 1998
Mild asthmatics	Exhaled NO			Exhaled NO is correlated with degree of airway hyperresponsiveness. Asthmatics who were treated with steroids had exhaled NO levels comparable to control subjects, although airways hyperresponsiveness could still be demonstrated.	Dupont et al., 1998
Healthy and asthmatic adults	Effect of NO on hypoxemia during methacholine-induced bronchoconstriction	80 ppm	10 min after methacholine inhalation	NO inhalation worsens desaturation during bronchospasm in humans after methacholine inhalation.	Takahashi et al., 1998
Methemoglobin					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
Healthy adults n=12	Systolic and diastolic blood pressure, heart rate, peripheral oxygen saturation, methemoglobin, biochemistry and cell count	40 ppm	2 hr	No changes in systolic or diastolic blood pressure, heart rate, oxygen saturation, blood cell counts, blood chemistry, except for a small decrease in platelets and aspartate amino transferase. Methemoglobin increased with NO inhalation, but remained in the normal range.	Luhr et al., 1998
Healthy adults n=5	Blood methemoglobin	32, 64, 128, 512 volumes per million	Sampling at 10 min intervals for 3 hr; or until methemoglobin exceeded 5% of total Hb	Inhalation of up to 128 volumes per million of NO does not result in clinically significant methemoglobinemia. Maximum methemoglobin levels likely are reached within 3 to 5 hr from the start of NO inhalation.	Young et al., 1994

Sickle cell Anemia					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
Adult sickle cell anemia patients n=7 Healthy adults n=5	Oxygen affinity of sickle cell erythrocytes	80 ppm	2 hr	Inhaled NO did not alter oxygen affinity of erythrocytes in either normal or sickle cell patients. Methemoglobin increased with NO inhalation, but remained within the normal range.	Gladwin et al., 1999
Oxidant Injury					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
Cell membrane lipids	Influence of NO on membrane lipid peroxidation induced by superoxide, hydrogen peroxide, and peroxynitrite			Relative rates of production and steady; state concentrations of superoxide and NO, the cellular and anatomical sites of production of superoxide and NO, and the dominant operative mechanisms of oxidant damage in tissues at the time of superoxide and NO production influence expression of the differential oxidant injury-enhancing and protective effects of NO.	Rubbo et al., 1994
Human low density lipoprotein (LDL)	Effect of NO on LDL oxidation			Continuous generation of NO can inhibit LDL oxidation, while NO and superoxide together lead to increased oxidant production.	Hogg et al., 1993
Sprague-Dawley rats exposed to 95% oxygen	Influence of inhaled NO on oxygen toxicity	10, 100 ppm	60 hr, 120 hr	Inhalation of 100 ppm NO improved survival of rats simultaneously inhaling 95% oxygen.	Nelin et al., 1998
Wistar rats males	Effect of NO on oxidant-induced lung injury using inhaled NO and inhibition of endogenous NO	10, 100 ppm	40 hr, concurrently with either 21% or 100% O2	Inhalation of 10 ppm NO reduced oxygen-induced injury, while 100 ppm NO had no effect on some endpoints, and increased the effect on others. Neither concentration of NO improved survival time with 100% oxygen inhalation, while inhibition of endogenous NO production reduced survival time, suggesting that endogenous production of NO is protective of oxidative damage.	Garat et al., 1997

Systemic Effects					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
Cats	Effects of inhaled NO on vasoactivity, leukocyte-endothelial cell interactions, and endothelial dysfunction in NO depleted, post-ischemic and septic vessels.	0, 20, 80 ppm		Inhaled NO was effective as an anti-adhesive, anti-vasoconstrictive and anti-permeabilizing molecule in NO-depleted tissues in the intestinal tract microvasculature. It was not effective in normal microvessels or in vessels that had an abundance of NO.	Fox-Robichaud et al., 1998
Nonresponders					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
ARDS patients who had received NO therapy	ABO blood group and response to inhaled NO	5-40 ppm		Reviewed medical records of hospitalized ARDS patients who had received inhaled NO therapy. Patients with blood group B or AB were less likely to have a significant improvement in oxygenation with NO inhalation, compared to patients with blood groups A or O.	Weimann et al., 1998
Interactions					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
Nonsmoking, healthy adults n=16	Exhaled NO	Ambient		Baseline exhaled NO = 7-43 ppb; mean = 28 ± 5 ppb when air pollution was low. With ambient air pollution exposure exhaled NO ranged from 5-60 ppb, and was positively associated with ambient NO. Exposure during early morning hours to high levels of air pollution was associated with increased exhaled NO, which persisted for up to 5 hours.	Van Amsterdam et al., 1999

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
18 healthy nonsmokers, 25-50 yrs 4 smokers, 22-24 yrs	Exhaled NO	Outdoor ambient on 4 days: 4, 30, 138 and 246 $\mu g/m^3$		On the two days with the highest outdoor air pollution, exhaled NO was increased significantly, 67-78%, above the mean baseline value assessed on four days with virtually o outdoor air pollution. Endogenous NO in exhaled air is increased on days with high outdoor air pollution.	Steerenberg et al., 1999
Healthy adults 41 smokers and 73 age-matched nonsmokers, all with normal lung function	Exhaled NO	Effect of cigarette smoke in smokers, and NO and CO inhalation in non-smokers on exhaled NO		In smokers, smoking a single cigarette decreased exhaled NO. Inhalation of NO and CO in nonsmokers had no effect on exhaled NO in normal subjects. The reduced NO after smoking a cigarette may contribute to the increased risk of respiratory disease in smokers.	Kharitonov et al., 1995
Normal adults during symptomatic upper respiratory tract infections and during recovery 3 weeks later n=18	Exhaled NO			Exhaled NO was 315±57 ppb during symptomatic upper respiratory tract infection, and 87±9 ppb 3 weeks later during recovery. The recovery value was in the same range as 72 age-matched normal control subjects, 88±3 ppb. The findings suggest that symptomatic upper respiratory tract infections markedly increase exhaled NO, and may reflect induction of an antiviral effect.	Kharitonov et al., 1995
Cultured bovine cerebellum cells	NO synthase activity	50-400 µM		Effects of NO were concentration dependent and reversible. NO production may be regulated by a direct effect of NO on the activity of NO synthase.	Rengasamy and Johns, 1994

Species/Subject	Endpoints	[NO]	Exposure Duration	Results	Reference
Murine macrophage cell line	Effect of NO generators and inhibitors on NO synthase activity in response to interferon-gamma plus lipopolysaccharide.			Results suggest that NO synthase activity is affected by availability of induced NO.	Assreuy et al., 1993
Bovine aortic endothelial cells	Role of NO as a negative feedback modulator of endothelial cell function through inhibition of NO synthase in vascular endothelial cells.			Pretreatment of cells with NO-donor agents caused a marked inhibition of endothelial NO biosynthesis in response to bradykinin and increased fluid shear or flow. Results suggest that NO plays an important negative feedback regulatory role on endothelial NO synthase and therefore, vascular endothelial cell function.	Buga et al., 1993
Epidemiology					
Species/Subject	**Endpoints**	**[NO]**	**Exposure Duration**	**Results**	**Reference**
Children	Croup	Ambient: Monthly averages 8 to 106 µg/m^3; highest daily average 450 µg/m^3	24 months	For the months of September through March, peak manifestation season for croup, there was a weak but statistically significant association between daily mean NO and NO_2 concentrations and incidence of croup.	Rebman et al., 1991
Humans	Hospital admissions for cardiac and cerebrovascular diseases	Ambient	1987-1989	Emergency room visits for ischemic cardiac diseases were significantly associated with ambient levels of NO.	Ponka and Virtanen, 1996
Humans	Emergency room visits for asthma	Ambient:	three years	Statistically significant association between emergency room visits for asthma and ambient NO concentration. The effect was greatest among working age adults, and secondarily among the elderly, although there was also a significant relationship for children.	Ponka, 1991

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