Nitric oxide (NO), a highly reactive gas produced by the enzymatic conversion of arginine to citrulline by a family of enzymes known as NO synthases (NOS), may be important in several physiologic processes, including host defense and BP regulation.\textsuperscript{1-3} Cigarette smoking has been associated with a number of adverse health consequences, including an increased incidence of respiratory tract infections and atherosclerosis.\textsuperscript{4-9} Consistent with the concept that NO production is disordered by cigarette smoke, NO has been reported to be reduced in the exhaled air of cigarette smokers.\textsuperscript{10-13}

We hypothesized that exhaled NO would be decreased in cigarette smokers but increased after smoking cessation. To test this hypothesis, exhaled NO was measured prior to and after smoking cessation. Consistent with results of previous studies,\textsuperscript{10-13} exhaled NO was found to be decreased in cigarette smokers. After smoking cessation, there was a rise in exhaled NO after 1 week and a further rise within 8 weeks to approximate the NO levels in normal nonsmokers.

**Materials and Methods**

**Subjects**

Normal nonsmoking control subjects (n=23; 15 male and 8 female; age, 38±3 years) were recruited from clinic personnel at the University of Nebraska Medical Center. Current cigarette smokers (n=25; 15 male and 10 female; age, 38±2 years) were recruited from the Smoking Cessation Clinic. All smoked ≥20
cigarettes per day and were heavy smokers (30.0±1.2 cigarettes per day). None of the subjects had a history of asthma, which is known to increase exhaled NO.10 None of the subjects were receiving any long-term medications, including bronchodilators.

After measuring exhaled NO and exhaled carbon monoxide (CO) levels (Bedford Scientific Ltd; London, UK), the smokers were counseled as a group, given nicotine patches, and asked to return after 1 week. Nineteen subjects returned. Fourteen were not smoking by self-report, which was confirmed by exhaled CO measurements. Five continued to smoke. The subjects again had their exhaled NO measured, were given group counseling, and were asked to use the nicotine patches for another 7 weeks. Ten subjects returned. All were abstinent from smoking by self-report, which was confirmed by exhaled CO measurements.

**Measurement of Exhaled NO**

The study was approved by the Institutional Review Board of the University of Nebraska Medical Center. Exhaled NO measurements were performed using a chemiluminescence analyzer (Sievers model 270B; Sievers; Boulder, Colo) as previously described.13 Samples were introduced into the analyzer at a flow rate of approximately 0.6 L/min through a 40-cm, narrow-bore, Teflon-coated tube, and responses were recorded on a chart recorder. The NO analyzer was calibrated at least daily using NO at a concentration of 113 parts per billion (ppb) in nitrogen (Scott Specialty Gases Inc; Plumsteadville, Pa). Response time of the analyzer with the recorder was 0.45±0.01 s from the initial deflection on the chart recorder to the peak when using the calibration gas at 113 ppb. Ambient NO was measured daily and was always <20 ppb.

After obtaining informed consent, the subject was asked to perform three maneuvers.13 First, with nose clips in place, the subject was asked to perform a slow vital capacity maneuver directly into the tubing connected to the analyzer.15,16 Second, the subject was asked to repeat the maneuver into a 5-L polyvinylfluoride bag (Tedlar; SKC; Eighty Four, Pa). Third, the patient was asked to exhale into a nasal mask (Respironics; Monroeville, Pa) connected to a Tedlar bag. The exhaled air collected in the bags was analyzed within 5 min. To ensure that NO did not react with the materials, the NO calibration gas was passed through the tubing, connections, the Tedlar bag, or through the nasal mask and compared with the NO calibration gas introduced directly into the NO analyzer. No decrease was seen.

**Statistics**

Data are reported as mean±SEM. Statistical comparisons between groups were performed using the two-tailed Student’s t test with a paired test used for paired data. Data from the three methods of measuring exhaled NO were compared using linear regression. Significance was defined as p<0.05.

**RESULTS**

**Baseline Levels of Exhaled NO**

Peak oral, mean oral, and nasal NO levels in the normal subjects and smokers approximated those previously reported.10,11,13 Exhaled NO was decreased in the exhaled air of cigarette smokers compared with normal nonsmokers whether measured by the peak oral (31.4±3.4 vs 105.5±8.4 ppb), mean oral (5.7±0.4 vs 14.6±1.1 ppb), or nasal techniques (25.0±1.9 vs 47.1±4.9 ppb, p<0.0001, all comparisons). There was good correlation between the peak oral and mean oral methods (r=0.715, p<0.0001), the mean oral and nasal methods (r=0.613, p<0.0001), and significant correlation between the peak oral and nasal methods (r=0.359, p=0.0105).

**Exhaled NO Levels in Smokers 1 Week After Cessation**

Nineteen subjects returned 1 week after begin¬ning treatment with nicotine patches. Fourteen subjects were not smoking by self-report, which was confirmed by an exhaled CO level of ≤2 ppm. Exhaled NO rose in these 14 subjects whether measured by the peak oral method (Fig 1, 63±7 vs 31±3 ppb, p=0.0029), mean oral method (Fig 2, 9.0±0.8 vs 5.7±0.4 ppb, p=0.0004), or the nasal method (Fig 3, 41±5 vs 25±2 ppb, p=0.0025). However, no increase was seen in the five subjects who had not successfully quit smoking (Figs 1-3, p>0.05, all comparisons). The exhaled NO levels were still depressed compared with the normal nonsmoking control subjects when measured by the peak oral method (63±7 vs 106±5 ppb, p=0.0192) or mean oral method (9.0±0.8 vs 14.6±1.1 ppb, p=0.0004). However, the exhaled nasal NO level had risen to a level that was not significantly different than in normal nonsmokers (41±5 vs 47±5 ppb, p=0.1532).

**Exhaled NO Levels in Smokers 8 Weeks After Cessation**

Ten smokers returned 8 weeks after smoking cessation and 1 week after stopping nicotine replacement. All were nonsmoking by self-report, which was confirmed by exhaled CO levels of ≤2 ppm. Exhaled NO levels further increased compared to 1 week after smoking cessation when measured by the peak oral method (Fig 4, 100±9 vs 63±7 ppb, p=0.0006) or mean oral method (Fig 4, 10.3±0.6 vs 9.0±0.8 ppb, p=0.043). Although the nasal exhaled NO rose compared to week 1 (Fig 4, 47±6 vs 41±5 ppb), the increase was not statistically significant (p=0.1932). Both the oral and nasal exhaled NO levels did not differ 8 weeks after smoking cessation compared to the normal nonsmoking control subjects (Fig 4, p>0.05, both comparisons). However, the mean oral NO was still slightly lower than the normal nonsmoker mean oral NO (Fig 4, 10.3±0.6 vs 14.6±1.1 ppb, p=0.018).
FIGURE 1. Peak oral exhaled NO measured in smokers before and 1 week after smoking cessation. Successfully quitting smokers are represented on the left and unsuccessful smokers are represented by on the right. Peak exhaled NO increased in the successful former smokers (p=0.0029), but not in the unsuccessful smokers.

DISCUSSION
This study demonstrates that exhaled NO is decreased in cigarette smokers and rises after smoking cessation. This increase occurred whether the exhaled NO was measured by either a peak oral, mean oral, or nasal technique. Eight weeks after smoking cessation, the exhaled NO levels approximated the levels of normal nonsmoking control subjects. Given the limitations of the small number of smokers who successfully quit at 8 weeks, future studies with larger sample sizes are warranted to confirm these observations.
weeks in the present study, these results suggest that the effects of cigarette smoking on exhaled NO are reversible.

Cigarette smoke may inhibit NO production by multiple mechanisms. Cigarette smoking causes a transient decrease in exhaled NO that returns to baseline levels within 15 min. This transient effect is consistent with the known inhibition of NOS activity by NO, which is present in cigarette smoke in high concentrations. However, this transient decrease would not seem to explain the lower levels of exhaled NO in cigarette smokers that were ob-

![Figure 3](https://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21749/) Figure 3. Nasal exhaled NO measured in smokers before and 1 week after smoking cessation. Nasal exhaled NO increased in the successful former smokers (p=0.0025), but not in the unsuccessful smokers.

![Figure 4](https://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21749/) Figure 4. Exhaled peak oral (open triangles), mean oral (closed circles), and nasal NO levels (open circles) in smokers prior to smoking cessation (week 0) and 1 or 8 weeks after smoking cessation. Levels of normal, nonsmokers are given at the right for comparison.
served after abstinence for at least 8 h. This more sustained reduction would be more consistent with the reduction in levels of NOS, the family of enzymes responsible for the NO production. Consistent with this concept, exposure of murine lung epithelial cells to a smoke extract decreases the transcription of the inducible form of NOS (R.A.R., unpublished observations).

The anatomic source of the exhaled NO is not known. Several lines of evidence suggest that airway epithelial cell production of NO might contribute to exhaled NO. The high levels of NO in the exhaled air and the high reactivity of NO suggest that the cellular source likely resides within the airways. Studies in rodent and/or human lung epithelial cells demonstrate the presence of NOS(8, 15, 16, 19-24). Furthermore, direct measurements from intubated patients during cardiopulmonary bypass demonstrate NO values of >5 ppb, suggesting that a substantial portion of the orally exhaled NO is derived from the lower respiratory tract. These observations would seem consistent with reduction in exhaled NO by cigarette smoking where exposure of the constituents to the cigarette smoke would be expected to be highest along the respiratory tract epithelium.

The optimal method for measuring exhaled NO is unknown. Both the lower respiratory tract and upper respiratory tract are exposed to smoke during smoking. Therefore, smoking would be expected to be associated with decreased NO, whether measured by either oral or nasal methods. Furthermore, this study is consistent with previous studies in our laboratory that demonstrate that the three methods used to measure exhaled NO correlate, with the correlation being stronger for the oral techniques than either oral technique with the nasal technique.

Cigarette smoking is associated with a number of adverse health effects. Several of these adverse effects might potentially be explained by a reduction in NO release. For example, reduction of NO by airway cells is likely important in host defense because NO has been reported to inhibit replication of potential respiratory pathogens. Reduction of NO production might explain, at least in part, the increased incidence of lower respiratory tract infections observed in cigarette smokers. NO has also been implicated in regulation of inflammatory cell chemotaxis, inflammatory cell adhesion, and airway epithelial cell cilia beating. These effects are also relevant to airway host defense and might explain an increased incidence of respiratory tract infection in smokers.

Reduction in NO production by cigarette smoking may contribute to the adverse effects of cigarette smoking, such as an increased incidence of lower respiratory tract infection. However, these studies demonstrate that this reduction is reversible, illustrating another potential benefit of smoking cessation.

**References**