LOW LEVELS OF NITRIC OXIDE AND CARBON MONOXIDE IN ALPHA 1-ANTITRYPsin DEFICIENCY


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Running head: NO and CO in α1-AT deficiency

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ABSTRACT

Quantitation of exhaled nitric oxide (NO) and carbon monoxide (CO) has been proposed to noninvasively measure markers of airway inflammation. We hypothesized that exhaled carbon monoxide (CO) is increased in individuals with alpha-1-antitrypsin (α1-AT) deficiency, who have lung inflammation and injury related to oxidative and proteolytic processes. Nineteen individuals with α1-AT deficiency, 22 healthy controls, and 12 patients with non-α1-AT deficient COPD had NO, CO, CO₂ and O₂ measured in exhaled breath. Individuals with α1-AT deficiency had lower levels of NO and CO than control or COPD individuals. α1-AT deficient and COPD patients had lower exhaled CO₂ than controls, although only α1-AT deficient patients had higher exhaled O₂ than healthy controls. Nitric oxide was correlated inversely with exhaled O₂ and directly with exhaled CO₂, supporting a role for NO in regulation of gas exchange. Exhaled gases were not significantly related to corticosteroid use or lung function. Demonstrating lower than normal CO and NO levels may be useful as an additional noninvasive method to evaluate for α1-AT deficiency in individuals with severe, early onset obstructive lung disease.

Keywords: alpha-1-antitrypsin deficiency, carbon monoxide, nitric oxide, airway inflammation, chronic obstructive pulmonary disease.
INTRODUCTION

Individuals with alpha-1-antitrypsin (α₁-AT) deficiency who are homozygous for the Z α₁-AT allele (PI*ZZ phenotype) are at an increased risk for the development of early-onset emphysema due to the lack of protection provided by the enzyme against parenchymal destruction mediated by neutrophil elastase (3). Bronchoalveolar lavage demonstrates an increased number of neutrophils in the lower airways of subjects with α₁-AT deficiency adding oxidative stress to the imbalance between the protective and proteolytic enzymes in the lower airways (16). A significant degree of airway inflammation in α₁-AT deficiency is evidenced by increased sputum levels of myeloperoxidase and leukotriene B₄ (14, 15). Thus, oxidative stress and inflammation ultimately add to the lung disease associated with α₁-AT deficiency.

Measuring alterations in exhaled monoxide gases has been proposed as a non-invasive method to assess surrogate markers of airway inflammation and oxidative stress. For example, elevated concentrations of nitric oxide (NO) have been demonstrated in the exhaled gases of individuals with a variety of inflammatory lung diseases such as asthma, bronchiectasis, and, occasionally, chronic obstructive pulmonary disease (COPD) (18). Increases in exhaled NO in inflammatory lung diseases have been linked to increased expression of nitric oxide synthase 2 (NOS2) in the human airway (11). NOS2 expression is increased by inflammatory cytokines, and thus may serve as a sensitive indicator of lung inflammation (12). In this context, it is surprising that exhaled NO levels of
individuals with PI*ZZ $\alpha_1$-AT deficiency are lower than NO levels of healthy controls, or of individuals with PI*M heterozygous phenotypes or non-$\alpha_1$-AT deficient COPD (22).

Recently, exhaled carbon monoxide (CO) has also been proposed as a marker of lung inflammation (18). Produced by heme oxygenases (HO), CO is increased in the exhaled breath of individuals with smoking related COPD (23). Based upon this, we hypothesized that exhaled CO is increased in $\alpha_1$-AT deficiency and that levels may be related to the severity of lung disease. To evaluate this, the exhaled gases including NO, CO, CO$_2$ and O$_2$, were measured in individuals with $\alpha_1$-AT deficiency in comparison to healthy controls and individuals with smoking-related COPD.
METHODS

Study Population. The study population included individuals with α₁-AT deficiency, COPD related to previous cigarette smoke exposure, and healthy controls. Individuals with PI*ZZ α₁-AT deficiency and serum α₁-AT levels below the protective threshold value of 11 µMol/l were identified at an educational patient-oriented meeting organized by the Cleveland Clinic Foundation. Healthy control individuals were identified by absence of pulmonary symptoms or history of pulmonary disease. COPD diagnosis was based on American Thoracic Society guidelines (1). Exclusion criteria included current cigarette smoking, asthma, recent respiratory tract infection, and exacerbation of lung disease within the previous six weeks.

The study was approved by the Institutional Review Board and informed consent was obtained from all volunteers.

Offline Collection and Measurement of Exhaled Gases. As previously described (20), exhaled gases were obtained by an offline method in agreement with ATS recommendations for exhaled NO determination. Briefly, individuals inhaled NO free air to total lung capacity and exhaled against 10 cm H₂O pressure to meet the ATS recommended flow rate of .35 l/s, into a Mylar collection bag (Physiological Measurement Systems, Bay Village, OH). All individuals were seated at rest for at least 15 min before gases were collected. Exhaled CO and CO₂ were measured in the exhaled gases with a Siemens Ultramat 6 infrared analyzer (Karlsruhe, Germany) that was adapted for use in this study. The analyzer was calibrated daily using CO free gas and a
gas with a known CO and CO$_2$ concentration. The analyzer was sensitive to a concentration of 100 ppb for CO and 0.1% for CO$_2$. Absorbed wavelengths for CO and CO$_2$ are characteristic and separable to the individual gases, so that CO$_2$ interference with CO does not occur (20). NO concentrations were determined using a chemiluminescence analyzer (Sievers Instruments, Boulder, CO). A Teledyne UFO-130 micro-fuel oxygen sensor (City of Industry, CA) was used for determination of exhaled O$_2$ levels. The oxygen analyzer was calibrated using zero air, followed by high gain calibration with 100% O$_2$ (Praxair, Cleveland, OH). Zero air was prepared by passing ultrapure nitrogen (99.999% pure nitrogen; PraxAir, Cleveland, OH) through a NuPure II Eliminator room temperature purifier for inert gases (Manotick, Ontario, Canada). The gas purifier reduces gaseous impurities to concentrations of less than 1 ppb for O$_2$, CO$_2$, CO, hydrogen dioxide, hydrogen, and methane. Purified gas was then collected and used as a zero calibration gas for the analyzers.

**Statistical Analysis.** Quantitative data are summarized as mean ± SE; categorical data are summarized by frequencies. Associations between pairs of variables are described by Pearson's correlation coefficient and a test for non-zero correlation. Two-tailed $t$ test statistics, Chi square, analysis of variance, and ANOVA on ranks were utilized where appropriate, with the Bonferroni correction being applied to the significance criterion once pairwise comparisons were made among the study groups. All tests were performed at individual significance levels of alpha 0.05.
RESULTS

Clinical characteristics. The characteristics of the study population are shown in Table 1. Patients with COPD were significantly older than individuals with α1-AT deficiency, and both groups were significantly older than healthy controls (p < 0.001). Individuals with COPD had a significantly longer smoking history than α1-AT deficient individuals (p < 0.001). Inhaled corticosteroid use was similar in both groups (p = 0.42), as was percent predicted FVC (p = 0.92), FEV1 (p = 0.91), and DLCO (p = 0.86). Four patients were on supplemental oxygen (α1-AT deficiency [n=2] and COPD [n=2]). For analyses of correlation between exhaled O2 and CO2 and exhaled O2 levels, individuals on supplemental oxygen were excluded.

Exhaled Gases. NO levels were lower in α1-AT deficient patients than in healthy controls or COPD patients (Table 2, Figure 1). NO did not correlate with lung function in α1-AT deficiency or COPD patients (all p > 0.1). However, NO correlated inversely with exhaled O2 (r = -0.575, p = 0.015) and directly with CO2 in the α1-AT deficient (r = 0.465, p = 0.044) (Figure 2). In contrast, NO was unrelated to exhaled O2 or CO2 in both the control and COPD groups.

The linear fit of the NO-O2 and NO-CO2 data reveals the following relationships between the exhaled gases:

\[
[\text{NO}] = 40 - \frac{[\text{O}_2]}{0.5} \quad (1)
\]

\[
[\text{NO}] = 0.7 + \frac{[\text{CO}_2]}{0.6} \quad (2)
\]
Like NO, CO levels were lower in α₁-AT deficiency than in the controls or individuals with COPD (Table 2, Figure 1). Carbon monoxide levels did not correlate with lung function or any other exhaled gas (all p > 0.1).

For both α₁-AT deficient and COPD patients, exhaled CO₂ levels were lower than controls (Table 2, Figure 1). Exhaled O₂ levels were only higher in α₁-AT deficiency (Table 2, Figure 1). Exhaled CO₂ correlated inversely with O₂ in all study groups, reflecting the relationship between O₂ uptake and CO₂ release from the lung (all p < 0.001) (Figure 3). Lung function did not correlate with levels of either of the two gases (all p > 0.1). Age did not correlate with exhaled gas values or lung function in any of the study groups (all p > 0.05), which is consistent with previous observations(7).

Interestingly, the linear fit of the O₂ - CO₂ data reveals different relationships between the exhaled gases among the different groups (analysis of covariance, p=0.001).

For controls:

\[ [O_2] = 19.6 - \frac{[CO_2]}{1.6} \]  

For α₁-AT deficiency:

\[ [O_2] = 20.7 - \frac{[CO_2]}{0.9} \]  

For COPD:

\[ [O_2] = 21.3 - \frac{[CO_2]}{0.8} \]
The different slope of the fitted lines for control and obstructive lung disease populations suggests that the $O_2$ uptake and $CO_2$ release from the lungs of individuals with obstructive lung disease maybe less efficient than in controls or that metabolic consumption of $O_2$ is altered in obstructive lung disease.

**Effect of Treatment on Exhaled NO and CO.** Previous studies have shown that inhaled corticosteroids (ICS) lower exhaled NO (19) and CO (31). To determine if the lower levels of NO and CO in individuals with $\alpha_1$-AT deficiency were related to steroid use, we evaluated individuals by type of therapy. NO levels were similar in $\alpha_1$-AT deficient individuals irrespective of inhaled corticosteroid use (NO [ppb]: – ICS 4.7 ± 0.8, + ICS 5.9 ± 1; $p = 0.4$). Similarly, COPD individuals on ICS had NO levels similar to those not receiving steroids (NO [ppb]: – ICS 13 ± 3.2, + ICS 16.5 ± 1.6; $p = 0.41$). CO was similar in the $\alpha_1$-AT deficiency group with or without ICS use (CO [ppm]: – ICS 0.6 ± 0.2, + ICS 0.3 ± 0.06; $p = 0.22$), and in the COPD group with or without ICS use (CO [ppm]: –ICS 1.6 ± 0.4, + ICS 1.5 ± 0.6; $p = 0.95$). It is possible, however, that due to the small number of patients in each group a lack of effect may be due to lack of statistical power. Although there was no difference in the exhaled gases of the steroid naïve versus the steroid treated groups, reanalysis of data was performed excluding those individuals receiving corticosteroids. The results were similar to those seen in the whole study population (NO [ppb]: $\alpha_1$-AT deficiency 4.7 ± 0.8, control 8.6 ± 0.6, COPD 13 ± 3.2, $p = 0.002$; CO [ppm]: $\alpha_1$-AT deficiency 0.6 ± 0.2, control 1.3 ± 0.11, COPD 1.6 ± 0.4, $p = 0.014$).
Exhaled NO and CO levels of $\alpha_1$-AT deficient individuals receiving augmentation therapy were similar to levels of those who were not (NO [ppb]: augmentation 5.7 ± 1, no augmentation 5.2 ± 1, $p = 0.73$; CO [ppm]: augmentation 0.3 ± 0.05, no augmentation 0.6 ± 0.2, $p = 0.19$).
DISCUSSION

The results of this study show that exhaled gases of α₁-AT deficient individuals are markedly altered when compared to healthy controls, and unexpectedly, as compared to individuals with non-α₁-AT deficient COPD. Exhaled CO and NO are lower in patients with α₁-AT deficiency than healthy controls and individuals with COPD. Further, CO₂ levels are lower than controls in both α₁-AT deficiency and COPD, while exhaled O₂ in α₁-AT deficiency is higher than in controls or COPD. Alterations in airflow or minute ventilation in COPD and α₁-AT deficient patients may be the cause of the decreased CO₂ seen in these patients, which had the same degree of pulmonary dysfunction, as compared to healthy controls. In contrast to the parallel change in CO₂, derangements of CO and NO where distinct between COPD and α₁-AT deficient groups. Hence, minute ventilation or alterations in airflow are not likely causes of the changes seen.

Several potential mechanisms may account for the decreased NO in α₁-AT deficiency, including increased consumption or decreased production of NO. Nitric oxide is consumed by its interaction with superoxide produced during neutrophil activation (17), which has been proposed as one mechanism for the low exhaled NO of patients with cystic fibrosis (2, 5, 29). Neutrophil predominance in α₁-AT deficiency airways with increased superoxide production may increasingly consume NO. In addition, neutrophil enzymes such as myeloperoxidase (MPO) consume nitrite (NO₂⁻) (13), which is considered a storage pool of NO in the airway and another source of exhaled NO.
Depletion of NO\textsubscript{2} may thus contribute to the decrease in exhaled NO (9). The combination of NO with superoxide, or NO\textsubscript{2} consumption by MPO both lead to reactive nitrogen species formation, e.g., peroxynitrite, which may further worsen inflammation and lung injury. Individuals with COPD also have neutrophilic influx and higher neutrophil numbers in the airways (28). However, it is possible that due to the relatively unopposed effects of neutrophilic proteolytic activity seen in $\alpha$-AT deficiency, a higher degree of NO consumption may still occur. Interestingly, polymorphisms in the NOS3 gene have been associated with severity of lung disease in $\alpha$-AT deficient patients (24). Although mutation in this site of NOS3 has not been shown to affect the activity or turnover of protein (8), other unrecognized mutations may affect the activity of the enzyme and consequently NO production. In contrast to low NO in $\alpha$-AT deficiency, COPD patients have high levels of NO. Thus, the finding of lower than control values of NO in individuals with obstructive lung disease may suggest a genetic cause for airflow limitation, including $\alpha$-AT deficiency, cystic fibrosis or primary ciliary dyskinesia (18). On the other hand, there are many potential explanations for the difference in NO levels including distribution of lung destruction, inflammatory cell concentration and/or type and extent of airways disease, which may have little direct relationship to $\alpha$-AT deficiency.

Although evidence supports a primary airway source of exhaled NO, there is considerable evidence suggesting that alveolar production is a significant source of exhaled CO (18). Tissue expression of inducible HO-1 predominantly in alveolar macrophages, and constitutive HO-2, predominantly in lung parenchyma, is increased in
cigarette smoke-exposed lungs irrespective of COPD, as compared to non-smoke exposed lungs (21). This suggests that HO is one source of the increased exhaled CO seen in COPD. Carbon monoxide levels decrease in asthma immediately after experimental antigen challenge, perhaps due to decreased diffusion into gas space from lung tissues, also supporting an alveolar source for the gas (20). In this context, impaired diffusion and alveolar destruction are likely mechanisms for the low exhaled CO in patients with emphysema due to α₁-AT deficiency. This may not be as prominent in the COPD population, a more heterogeneous group in terms of their pathologic manifestations ranging from chronic bronchitis to emphysema. On the other hand, a polymorphism in the HO-1 gene promoter is associated with susceptibility to the development of cigarette-smoke related emphysema (30). The polymorphism leads to diminished HO-1 response to oxidative stress (e.g., as with cigarette-smoke exposure). The relationship of HO polymorphisms to lung disease in general is unknown, but CO administration has been shown to exert protective anti-inflammatory effects in experimental models of lung injury, suggesting that a decrease in CO production could contribute to oxidative stress and development of emphysema (25, 26).

Lower exhaled CO₂ levels in α₁-AT deficiency and COPD likely reflect the impairment in gas exchange associated with obstructive lung disease, although we cannot exclude some degree of hyperventilation secondary to the presence of obstructive lung disease causing a decrease in exhaled CO₂. Exhaled O₂ is significantly higher in α₁-AT deficiency than in controls or COPD patients, and inversely related to NO. Parenchymal destruction associated with emphysema may be a less likely cause for the decreased O₂ uptake, since COPD individuals had similar lung function to those with α₁-AT
deficiency. However, the lower than normal NO in $\alpha_1$-AT deficiency may be detrimental to $O_2$ uptake. Nitric oxide promotes pulmonary arterial vasodilatation and plays a central role in ventilation-perfusion matching (4, 6). Furthermore, NO may play an important role in oxygen uptake and delivery to peripheral tissues by regulating vascular tone in response to tissue oxygen tension (10, 27). Thus, diminished NO in $\alpha_1$-AT deficiency may contribute to the derangements in ventilation/perfusion matching and tissue oxygenation leading to less $O_2$ uptake and higher exhaled $O_2$.

In conclusion, individuals with alpha-1-antitrypsin deficiency have low exhaled levels of NO and CO compared to healthy controls and patients with non-$\alpha_1$-AT deficient COPD. Although the precise mechanisms responsible for these findings remain unclear, the effects do not seem to be related to lung function or inhaled corticosteroid use.

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REFERENCES


FIGURE LEGENDS

FIGURE 1. Individuals with $\alpha_1$-AT deficiency have low levels of exhaled CO and NO in comparison to controls or COPD. Exhaled CO$\ _2$ is lower than controls in both $\alpha_1$-AT deficiency and COPD, although only $\alpha_1$-AT deficient individuals have higher exhaled O$\ _2$ levels than controls. Values represent median ± 25-75%, with dots representing outliers beyond 25-75%.

FIGURE 2. NO in $\alpha_1$-AT deficiency individuals is inversely related to exhaled O$\ _2$ (A) and directly related to exhaled CO$\ _2$ (B).

FIGURE 3. Inverse correlation between exhaled O$\ _2$ and exhaled CO$\ _2$ in all study populations. Healthy control (open squares/dotted line, $r = -0.755, p <0.001$), $\alpha_1$-AT deficiency (closed circles/solid line, $r = -0.960, p <0.001$), COPD (open circles/dashed line, $r = -0.970, p <0.001$).
Table 1. Clinical Characteristics of Study Population.

<table>
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<th>$\alpha_1$-AT deficiency</th>
<th>Control</th>
<th>COPD</th>
</tr>
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<tbody>
<tr>
<td><strong>N</strong></td>
<td>19</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td><strong>Age</strong> $^*$</td>
<td>50 ± 2</td>
<td>33 ± 2</td>
<td>64 ± 3</td>
</tr>
<tr>
<td><strong>Gender (M/F)</strong></td>
<td>8/11</td>
<td>13/9</td>
<td>8/4</td>
</tr>
<tr>
<td><strong>Serum alpha-1- anti-trypsin level (µmol/l)</strong></td>
<td>4.0 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cigarettes (pack/y)</strong></td>
<td>19 ± 3</td>
<td>0</td>
<td>60 ± 7</td>
</tr>
<tr>
<td><strong>Ever received</strong></td>
<td>11 (84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>augmentation therapy (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Inhaled steroids (%)</strong></td>
<td>12 (63)</td>
<td>5 (38)</td>
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<tr>
<td><strong>FEV$_1$ % predicted</strong></td>
<td>52 ± 8</td>
<td>53 ± 5</td>
<td></td>
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<tr>
<td><strong>FVC % predicted</strong></td>
<td>83 ± 5</td>
<td>82 ± 6</td>
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<tr>
<td><strong>DL$_{CO}$ % predicted</strong></td>
<td>62 ± 6</td>
<td>61 ± 5</td>
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All values mean ± SE, $^*$p < 0.001.
Table 2. Exhaled Gases in Study Groups

<table>
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<tr>
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<th>Control</th>
<th>COPD</th>
<th>pANOVA</th>
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<tbody>
<tr>
<td>NO ppb</td>
<td>5.5 ± 0.7</td>
<td>8.6 ± 0.6</td>
<td>14.5 ± 2.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CO ppm</td>
<td>0.4 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.5 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CO₂ %</td>
<td>2.7 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>3.1 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>O₂ %</td>
<td>17.7 ± 0.2</td>
<td>17.0 ± 0.1</td>
<td>17.0 ± 0.4</td>
<td>0.022</td>
</tr>
</tbody>
</table>

All values mean ± SE; pairwise comparison: * p < 0.01 vs. control, † p < 0.01 vs. COPD.
Figure 1
Figure 2

A) 

![Graph showing NO ppb vs. O2 %]

B) 

![Graph showing NO ppb vs. CO2 %]

Figure 2
Figure 3