Airway Blood Flow Reactivity in Healthy Smokers and in Ex-Smokers With or Without COPD*

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**Study objectives:** Cigarette smoking has been associated with impaired endothelium-dependent relaxation responses in the brachial and coronary arteries (endothelial dysfunction). The aim of the present study was to determine whether the airway circulation is also affected and whether pharmacologic treatment has an effect on endothelial function in patients with COPD.

*Methods and patients:* Airway blood flow (Q˙aw) responses to therapy with inhaled albuterol, which causes endothelium-dependent vasodilation, were measured with a noninvasive soluble-gas-uptake technique in age-matched healthy current smokers (n = 10), healthy ex-smokers (n = 10), ex-smokers with COPD (n = 10), and healthy lifetime nonsmokers. In the ex-smokers with COPD, the albuterol responsiveness measurement was repeated after 4 weeks of treatment with fluticasone/salmeterol and after a drug washout period of 4 or 8 weeks.

**Results:** The mean (± SE) baseline Qaw values ranged between 40.7 ± 3.9 and 50.9 ± 2.8 µL/min/mL anatomic dead space in the four groups (differences were not significant). The mean FEV1 was 53.4 ± 2.3% predicted in the ex-smokers with COPD. Albuterol inhalation increased mean Qaw significantly in lifetime nonsmokers (50.1 ± 8.3% predicted; p < 0.05) and healthy ex-smokers (37.2 ± 3.4% predicted; p < 0.05), but not in healthy current smokers (13.9 ± 3.2% predicted; difference was not significant) and ex-smokers with COPD (9.7 ± 4.5% predicted; difference was not significant). While fluticasone/salmeterol did not change Qaw significantly, it restored albuterol responsiveness (67.6 ± 11.1% predicted; p < 0.05) in the ex-smokers with COPD; this effect was no longer seen after the drug washout period.

**Conclusions:** Cigarette smoking is associated with a blunted vasodilator response to inhaled albuterol in the airway as an expression of endothelial dysfunction, with a partial recovery of albuterol responsiveness after smoking cessation in healthy ex-smokers but not in ex-smokers with COPD. In the latter group, combined glucocorticoid/long-acting β₂-adrenergic agonist treatment restores albuterol responsiveness. The role of endothelial dysfunction in the physiopathology of COPD remains to be examined.

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Key words: β₂-adrenergic agonists; bronchial circulation; COPD; endothelial function; glucocorticoids; smoking

Abbreviations: DME = dimethylether; DS = anatomic dead space minus the most proximal 50 mL; NO = nitric oxide; NOS = nitric oxide synthase; Qaw = airway blood flow; VD = anatomic dead space; VDME = helium-corrected dimethylether slope multiplied by the anatomic dead space minus the most proximal 50 mL

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Cigarette smoking is associated with attenuated endothelium-dependent relaxation responses in the systemic circulation.1-3 The airway circulation is part of the systemic circulation and can be expected to participate in the global endothelial dysfunction that is seen in smokers. This has thus far not been investigated, yet the airways are critically involved in the physiopathology of cigarette smoke-induced COPD.

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It has been well-established by in vitro and in vivo studies\textsuperscript{4–7} that $\beta_2$-adrenergic agonists cause vasodilation predominantly by endothelial nitric oxide synthase (NOS) activation and nitric oxide (NO) release, and $\beta_2$-adrenergic agonists are commonly used to investigate endothelium-dependent vasodilation. In the present study, we considered the response of airway blood flow ($Q_{aw}$) to inhaled albuterol as an index of endothelial function. Using this approach, we wished to determine whether cigarette smoking causes endothelial dysfunction in the airway by comparing healthy current smokers with age-matched healthy ex-smokers, ex-smokers with COPD, and healthy lifetime nonsmokers. Furthermore, we assessed the effect of airway therapy with an inhaled long-acting $\beta_2$-adrenergic agonist/glucocorticoid combination preparation on endothelial function in the subjects with COPD. We hypothesized that endothelium-dependent vasodilation is impaired in the airway of current and ex-smokers with or without COPD, and that airway treatment will restore endothelium-dependent vasodilation.

**Materials and Methods**

**Test Subjects**

Ten healthy current smokers, 10 healthy ex-smokers, 10 glucocorticoid-naïve ex-smokers with COPD, and 10 healthy lifetime nonsmokers were recruited for this study. The healthy current smokers and ex-smokers and the ex-smokers with COPD had to have a smoking history of at least 10 pack-years, and the ex-smokers must have quit smoking at least 1 year before the study. Healthy current smokers and ex-smokers and healthy lifetime nonsmokers had to be free of dyspnea, and had to have an FEV\textsubscript{1} of > 80% predicted and an FEV\textsubscript{1}/FVC ratio of > 0.7. The diagnosis of COPD required the presence of exertional dyspnea, an FEV\textsubscript{1} of < 75% predicted, and an FEV\textsubscript{1}/FVC ratio of < 0.7. At study entry, the subjects with COPD were clinically stable; they used short-acting $\beta_2$-adrenergic agonists and cholinergic antagonists as their usual airway medication. None of them was receiving oral airway medications or using domiciliary oxygen.

None of the subjects was receiving oral antiinflammatory medication. All subjects denied having experienced an acute respiratory infection for at least 1 month before the study, and no subject developed an acute respiratory infection during the study. All subjects denied having cardiovascular disease or taking vasoactive medications. The University of Miami Institutional Review Board approved the study protocol. All subjects provided written informed consent and received financial remuneration for their participation.

**Spirometry**

FEV\textsubscript{1}, FVC, and the FEV\textsubscript{1}/FVC ratio were determined (Spirovit SP10 spirometer; Welch-Allyn; Skaneateles Falls, NY). The tracing with the highest FEV\textsubscript{1} value of three maneuvers was analyzed. Predicted normal values were taken from the study by Crapo et al.\textsuperscript{8}

**Qaw**

A previously validated soluble inert gas uptake method was used to measure Qaw.\textsuperscript{9–11} The subjects first inhaled room air to total lung capacity. After exhaling 500 mL, they rapidly inhaled the same volume of a gas mixture from a bag (Teflon; DuPont; Wilmington, DE), consisting of 10% dimethylether (DME), 5% helium, and balance oxygen. After a predetermined breathhold time, the subjects then exhaled into a spirometer through a critical flow orifice to standardize the expiratory flow. During exhalation, the instantaneous concentrations of DME, nitrogen, and helium were measured at the airway opening with a mass spectrometer (Perkin-Elmer; Pomona, CA) along with the expired gas volume. The maneuver was performed with two breathhold times each of 5, 10, 15, and 20 s in random order. The helium-corrected decrease in DME concentration over time was obtained by least-squares fit, using the two measurements per gas for each of the four breathhold times. This was done in the expired volume fraction corresponding to the anatomic dead space minus the most proximal 50 mL (DS). Anatomical dead space (VD) was determined from the expired nitrogen concentration curve as described by Fowler and coworkers.\textsuperscript{12} From the helium-corrected DME slope multiplied by DS (VDME), the mean DME concentration in the DS (Q), and the solubility coefficient for DME in blood and tissue (a), Qaw was calculated using the Fick principle ($Q_{aw} = V_{DME}/a \times F_{DME}$). Qaw was normalized for VD, and expressed as microliters per minute per milliliter. Five to eight minutes were required for one Qaw determination.

**Protocol**

The subjects were asked to come to the research laboratory in the morning of the study day. They were instructed to abstain from ingesting alcoholic beverages or using phosphodiesterase-5 inhibitors the night before the study and from consuming coffee or caffeinated drinks in the morning of the study. Current smokers were instructed to abstain from smoking for at least 12 h before the test. The COPD subjects were asked not to use their regular inhaled short-acting bronchodilators or the study medication for at least 12 h before the study.

On visit 1, systemic BP, pulse, O\textsubscript{2} saturation, spirometry, and Qaw were measured in all subjects before and 15 min after the inhalation of 180 $\mu$g of albuterol from a metered-dose inhaler using a spacer. The subjects with COPD were then entered into a 12-week protocol consisting of three 4-week periods, with the measurements made at visit 1 repeated after each period (i.e., visits 2, 3, and 4). Using a single-blind crossover design, the COPD subjects were randomly assigned to a 4-week treatment period with an inhaled fluticasone/salmeterol combination (250/50 $\mu$g, respectively) every 12 h followed by a 4-week treatment with placebo, or vice versa (periods 1 and 2). This was followed by a 4-week washout period (period 3). Thus, half of the COPD subjects had not received fluticasone/salmeterol for 4 weeks and half had not received them for 8 weeks at visit 4.

**Statistical Analysis**

The data were analyzed using a statistical software package (JMP for Macintosh, version 4.0; SAS Institute, Cary, NC). Multifactorial analysis of variance was used to determine the overall differences among treatments followed by a paired t test to identify specific pair differences. Significance was accepted at $p < 0.05$. Values are presented as the mean ± SEM.
RESULTS

The subject groups were age-matched (Table 1). The amount of cigarette smoking was similar in the healthy current smokers (33.1 ± 6.0 pack-years) and healthy ex-smokers (30.0 ± 6.3 pack-years), and was greater in ex-smokers with COPD (52.0 ± 8.0 pack-years; \( p < 0.05 \)). On average, healthy ex-smokers had quit smoking 6 years before the study (range, 2 to 19 years), while ex-smokers with COPD had quit 3 years before the study (range, 1 to 12 years; difference was not significant). The baseline mean FEV\(_1\) (53.4 ± 2.3% predicted) was lower in COPD patients than in patients in the other groups (Table 1).

The mean baseline values for pulse, BP, and \( O_2 \) saturation were not different among the four groups. Likewise, there was no significant difference in the mean baseline Qaw among the groups, although the mean Qaw tended to be higher in healthy current smokers and in ex-smokers with COPD (Table 1).

Qaw Response to Albuterol

Since the pre-albuterol therapy mean Qaw values were comparable in the different groups, albuterol responsiveness was expressed as the percentage increase from the pre-albuterol therapy value. Therapy with inhaled albuterol increased the mean Qaw by 50.1 ± 8.3% in healthy lifetime nonsmokers (\( p < 0.05 \)) and by 37.2 ± 3.4% in healthy ex-smokers (\( p < 0.05 \)). In contrast, albuterol therapy failed to increase the mean Qaw in healthy current smokers (increase, 13.9 ± 3.2%; difference not significant) and in ex-smokers with COPD (increase, 9.7 ± 4.5%; difference not significant) [Fig 1]. There was no correlation between the magnitude of albuterol responsiveness, on the one hand, and the amount of smoking in current and ex-smokers or the elapsed time after smoking cessation in ex-smokers on the other.

Fluticasone/salmeterol treatment restored Qaw responsiveness to albuterol in the ex-smokers with COPD; the mean Qaw then increased by 67.6 ± 11.1% (\( p < 0.001 \)), which is comparable to the response observed in the healthy lifetime nonsmokers (Fig 1). Albuterol responsiveness was again blunted after the drug washout period in these subjects; the mean Qaw

![Figure 1. Qaw response to albuterol in healthy current smokers (n = 10), healthy ex-smokers (n = 10), ex-smokers with COPD (ie, pretreatment, posttreatment, postplacebo, and post-washout period) \( n = 10 \), and healthy lifetime nonsmokers (n = 10). Values are expressed as the mean (± SE) percentage change from the respective baseline value. * = \( p < 0.05 \) vs other COPD conditions; ** = \( p < 0.05 \) vs other groups except COPD post-treatment.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22042/)

Table 1—Demographics and Baseline Characteristics of Study Participants*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Healthy Current Smokers (n = 10)</th>
<th>Healthy Ex-Smokers With COPD (n = 10)</th>
<th>Healthy Nonsmokers (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>52 (46–60)</td>
<td>56 (46–62)</td>
<td>57 (46–64)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 7</td>
<td>Male 8</td>
<td>Male 6</td>
</tr>
<tr>
<td></td>
<td>Female 3</td>
<td>Female 2</td>
<td>Female 4</td>
</tr>
<tr>
<td>Pack-yr smoked</td>
<td>33.1 ± 6.0</td>
<td>30.0 ± 6.3</td>
<td>NA</td>
</tr>
<tr>
<td>Time elapsed after smoking cessation, yr</td>
<td>NA (2–19)</td>
<td>3 (1–12)</td>
<td>NA</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>79 ± 1</td>
<td>85 ± 1</td>
<td>84 ± 1</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>121 ± 1</td>
<td>108 ± 2</td>
<td>128 ± 2</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>75 ± 1</td>
<td>68 ± 1</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>FEV(_1), % predicted</td>
<td>102.2 ± 1.5</td>
<td>99.7 ± 1.2</td>
<td>53.4 ± 2.31</td>
</tr>
<tr>
<td>Qaw, μL/min/mL</td>
<td>46.4 ± 2.3</td>
<td>40.9 ± 3.8</td>
<td>50.9 ± 2.8</td>
</tr>
</tbody>
</table>

*Values are given as the mean (range) or mean ± SE. NA = not applicable.
†p < 0.05 vs healthy current smokers and ex-smokers.
‡p < 0.05 vs other groups.
FEV1 Response to Albuterol

Because the ex-smokers with COPD had lower pre-albuterol therapy FEV1 values than the other groups, the response to albuterol was expressed as the change in percent predicted. The FEV1 response tended to be greater in the ex-smokers with COPD than in the three healthy groups, but this did not reach statistical significance except before fluticasone/salmeterol treatment (Fig 2). In the ex-smokers with COPD, albuterol responsiveness was the same after fluticasone/salmeterol or placebo treatment, and after drug washout. There was no correlation between the albuterol responsiveness of Qaw and FEV1 at any time of measurement in the four subject groups.

The mean pre-albuterol therapy FEV1 tended to increase after fluticasone/salmeterol treatment with a return to baseline, but the changes did not reach the level of significance (before treatment, 1.81 ± 0.5 L; after treatment, 2.02 ± 0.2 L; after the washout period, 1.89 ± 0.1 L; differences were not significant among the three groups).

FIGURE 2. FEV1 response to albuterol in healthy current smokers (n = 10), healthy ex-smokers (n = 10), ex-smokers with COPD (ie, pretreatment, posttreatment, postplacebo, and post-washout period) [n = 10], and healthy lifetime nonsmokers (n = 10). Changes are expressed as the mean (± SE) percentage of predicted normal values.

Discussion

We conclude from this observation that (1) cigarette smoking is associated with a blunted vasodilator response to inhaled albuterol in the conducting airways, (2) the response remains attenuated after smoking cessation in ex-smokers with COPD, and (3) there is partial recovery of the response in healthy ex-smokers. We also found that a 4-week treatment with inhaled fluticasone/salmeterol restores the response in the ex-smokers with COPD, with the effect no longer detectable 4 to 8 weeks after withdrawing the drug.

The amount and duration of smoking were similar in the current and ex-smokers as was the time elapsed between smoking cessation and the study in the ex-smokers. There was no correlation among any of these three aspects of the smoking history and albuterol responsiveness in patients in any of the three smoking groups. Consequently, it cannot be determined from this investigation how much smoking is required for abnormal albuterol responsiveness to develop. Theoretically, blunted relaxation responses to albuterol could be related to abnormal vascular smooth muscle function or decreased stimulated endothelial NO release. Impairments of both endothelium-independent vascular relaxation (assessed with nitroglycerin, which acts directly on vascular smooth muscle) and endothelium-dependent vascular relaxation (assessed with β2-adrenergic agonists) have been reported, with the latter impairment being more consistent and of greater magnitude. Endothelial dysfunction probably results from exogenous oxidative stress by oxidants, and possibly from the nicotine contained in cigarette smoke and/or the endogenous production of oxygen-free radicals (ie, superoxide, hydrogen peroxide, and hydroxyl) as a host response to cigarette smoke. While the abnormal vasodilator response to inhaled albuterol in our study cannot conclusively be equated with endothelial dysfunction (as nitroglycerin responsiveness was not assessed), the results of several previous studies strongly support the interpretation that the blunted vasodilator response to albuterol reflected endothelial dysfunction. First, it has been well-established that in the systemic circulation, β2-adrenergic agonists cause vasodilation predominantly by endothelial NOS activation and NO release. Second, while it is not known whether endothelial NOS is expressed in the human airway vasculature, functional NOS activity and the involvement of NOS activation in β2-adrenergic agonist-induced vasodilation have been shown in the airway circulation of sheep, suggesting an endothelium-dependent mechanism. Third, β2-adrenergic
agonists have been used to assess endothelial function in other systemic vascular beds.\(^5\),\(^{16}\)

In our study, there was a difference in the vascular albuterol responsiveness between healthy ex-smokers and ex-smokers with COPD. In the former group, there appeared to be a partial recovery of the response, with a significant increase in mean $Q_{aw}$ but of a lesser magnitude than that in healthy lifetime nonsmokers.

The mean increase of $Q_{aw}$ after treatment with 180 $\mu$g of albuterol was 50% (lower confidence interval, 20%) in healthy lifetime nonsmokers. If one considers that an increase in $Q_{aw}$ of $<20\%$ signifies the presence of endothelial dysfunction, 3 of 10 healthy ex-smokers and 2 of 10 healthy lifetime nonsmokers had endothelial dysfunction. In contrast, using this criterion, 8 of 10 ex-smokers with COPD and 10 of 10 healthy current smokers had endothelial dysfunction. This indicates that the mechanisms underlying abnormal albuterol responsiveness differ between ex-smokers with and without COPD. Perhaps, endothelium-independent relaxation is also impaired in subjects with COPD.

We have previously shown\(^{11,17,18}\) that the vasodilator responsiveness to inhaled albuterol is blunted in lifetime nonsmokers with asthma and that the defect can be corrected by treating the patients with an inhaled glucocorticosteroid or leukotriene modifier, suggesting that the defect is a manifestation of the asthma-associated airway inflammation. COPD is also characterized by airway inflammation, albeit in a pattern that is different from that seen in asthma.\(^{19}\) Yet, albuterol responsiveness was restored in our COPD patients with fluticasone/salmeterol therapy as well, again showing that antiinflammatory treatment is effective and that ongoing airway inflammation in ex-smokers with COPD may have a role in the abnormal albuterol responsiveness. It would have been interesting to assess the effect of antiinflammatory treatment on albuterol responsiveness in healthy current and ex-smokers; this was not possible in the present study for ethical reasons.

This is the first reported investigation of $Q_{aw}$ in COPD patients. The airways as well as the alveolar structures are remodeled in COPD patients, presumably as a result of the neutrophilic inflammation that is characteristic of this disease.\(^{19-21}\) One would therefore expect that the airway vasculature is also included in the remodeling. However, a morphologic examination of the airway vasculature has failed to show significant changes in the number of vessels and in the proportion of the airway submucosa taken up by vessels in COPD patients, which is in contrast to the situation with asthma patients in whom increased vascularity of the submucosal structures is typically seen.\(^{19,22}\) Therefore, our finding that $Q_{aw}$ was not significantly higher in ex-smokers with moderately severe COPD than in age-matched healthy lifetime nonsmoking subjects is consistent with the morphologic observations.

Fluticasone/salmeterol treatment had no effect on the mean pre-albuterol therapy $FEV_1$ and the albuterol-induced change in $FEV_1$ in the subjects with COPD. The latter observation further supports the notion that airway inflammation affects airway and airway vascular smooth muscle function differently, possibly due to the fact that airway vascular smooth muscle responsiveness to albuterol involves the endothelium. We saw a similar dissociation between the effects of antiinflammatory therapy on $FEV_1$ and $Q_{aw}$ responsiveness to albuterol, which was previously seen in another study involving asthmatic patients.\(^{16}\) As in healthy and asthmatic subjects, albuterol had no effect on $VD$ and hence on the calculation of $Q_{aw}$ in the present study.\(^{17}\)

The number of subjects in each group was limited for technical reasons. However, we think that the observed differences were meaningful, as we have previously used similar sample sizes in studies demonstrating significant differences in $Q_{aw}$ and $Q_{aw}$ responsiveness in healthy and asthmatic subjects. The residual effects of salmeterol on $FEV_1$ and $Q_{aw}$ were unlikely in this study as the subjects were required to have stopped receiving fluticasone/salmeterol at least 12 h before coming to the laboratory. This requirement also eliminated the possibility that the results were influenced by the previously shown acute vasoconstricting effect of inhaled fluticasone in the airway.\(^{23}\)

Other studies\(^{24-28}\) have shown the presence of endothelial dysfunction in the pulmonary circulation of smokers and patients with COPD. Since cigarette smoke-induced COPD involves not only the lung parenchyma but the bronchial tree as well, it seemed important to us to also study relaxation responses in the airway circulation of smokers. Endothelial dysfunction in the pulmonary and airway circulation of smokers may or may not have a role in the development of COPD. However, the present investigation has shown that relaxation responses are blunted in smokers and subjects with COPD, presumably as an expression of vascular inflammation. The results also indicate that pharmacologic treatment can restore relaxation responses in COPD patients. Further studies will be needed to assess the possibility that endothelial dysfunction in the airway circulation has a pathogenetic role in COPD.

REFERENCES

1. Celermajer DS, Sorensen KE, Georgakopoulos D, et al. Cigarette smoking is associated with dose-related and poten-