

Evidence of remodeling in peripheral airways of patients with mild to moderate asthma: Effect of hydrofluoroalkane-flunisolide

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Background: We have shown previously that inflammation in asthma is not restricted to central airways but can also be demonstrated in peripheral airways. It is not clear whether inflammation of the peripheral airways is associated with structural changes and whether this remodeling process can be modulated by deposition of inhaled corticosteroids (ICSs). **Objectives:** To compare remodeling in peripheral and central airways and to investigate the effects of hydrofluoroalkane (HFA)-ICS on remodeling at these sites.

Methods: Transbronchial and endobronchial biopsies were obtained from 12 patients with mild to moderate asthma before and after a 6-week course of HFA-ICS (flunisolide). Total collagen deposition, expression of collagen III, TGF- β , and α -smooth muscle actin were examined by using Van Gieson staining and immunocytochemistry, respectively.

Results: Total collagen occupied 37.7% of the wall area of peripheral airways, compared with 54.5% of the wall area of central airways ($P = .04$). There was no significant difference in central versus peripheral airways for collagen III or α -smooth muscle actin immunoreactivity and in the number of TGF- β^+ cells in the submucosa. The only significant effect of HFA-flunisolide was a decrease in α -smooth muscle actin area in peripheral airways (13.4% vs 4.6%; $P = .01$) that correlated with the percentage increase in forced expiratory flow at 25% to 75% of vital capacity ($r_s = -1.00$; $P = .00$).

Conclusion: Our data show that there is a considerable degree of airway remodeling in peripheral airways in patients with asthma and confirm the inability of ICS to modulate collagen deposition and TGF- β expression. Treatment with HFA-flunisolide is associated with a significant decrease in the expression of α -smooth muscle actin in peripheral airways, which correlated with improvement in peripheral airway function. (*J Allergy Clin Immunol* 2005;116:983-9.)

Key words: Asthma, flunisolide, hydrofluoroalkane, inhaled corticosteroids, remodeling, small airway, peripheral airways

Asthma is defined by the presence of respiratory symptoms associated with variable airflow obstruction,

Abbreviations used

ATS: American Thoracic Society
FEF₂₅₋₇₅: Forced expiratory flow at 25% to 75% of vital capacity
HFA: Hydrofluoroalkane
ICS: Inhaled corticosteroid

airway hyperresponsiveness, bronchial inflammation, and airway remodeling. Airway remodeling refers to airway structural alterations and likely has clinical consequences. Epithelial shedding, goblet cell and submucosal gland hyperplasia, increased smooth muscle mass, subepithelial fibrosis, microvascular alterations, cartilage changes, and airway wall edema are the main histological features of asthmatic airways.

Subepithelial fibrosis is a component of remodeling widely described in central airways of subjects with asthma, ranging in severity from mild to severe.¹⁻³ The thickness of the reticular basement membrane has been correlated to airflow obstruction and increased airway hyperresponsiveness.^{2,4} Fibrosis results from an increased deposition of extracellular matrix proteins including different types of collagens, fibronectin, tenascin, lumican, and biglycan in the lamina reticularis, and may extend to the submucosal layer.⁵⁻⁹ The association of fibrosis observed in the submucosal layer with lung function and disease severity is a matter of controversy.^{10,11}

Evidence of remodeling has been previously reported in peripheral airways¹² obtained from autopsy asthmatic lung specimens. Increase in the thickness of adventitial, submucosal, and muscle layers has been demonstrated in asthmatic versus normal peripheral airways.^{13,14} James et al¹² have described an increase in reticular basement membrane thickness in peripheral cartilaginous and membranous airways. An increase in smooth muscle mass is also a common observation in asthmatic airways¹⁵ that has the potential to enhance bronchoconstriction.^{16,17} Accurate quantification of smooth muscle mass has not been performed in peripheral airways.

It is now well known that the airway inflammatory process occurs not only in central airways but also in peripheral airways and in lung parenchyma.^{18,19} In fact, CD3⁺ T cells and eosinophils infiltrate both peripheral and central airways in asthma to the same extent.¹⁸ Minshall et al¹⁹ and Taha et al²⁰ have shown that IL-5,

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IL-4, and eotaxin are highly expressed in peripheral airways and lung parenchyma compared with controls without asthma.

Corticosteroids are effective in achieving control of asthma. Corticosteroids reduce airway eosinophilic inflammation and expression of GM-CSF, IL-4, IL-5, IL-11, and IL-17.²¹⁻²³ To be effective, inhaled corticosteroids (ICSs) need to be deposited in the airways. In its first formulation, 19.7% of the inhaled dose of chlorofluorocarbon-flunisolide taken with a spacer device was deposited in the lungs.²⁴ The new hydrofluoroalkane (HFA) formulated flunisolide is delivered to the airways more effectively (68.3%) and is deposited preferentially in the distal airways.²⁴ Recently, we have shown that eosinophilic inflammation can be suppressed by HFA-flunisolide in both peripheral and central airways.²⁵ However, it is not clear whether corticosteroid deposition in peripheral airways results in sufficiently high drug concentrations to influence remodeling.

The current report forms part of a study that was designed to examine the efficacy of the anti-inflammatory effect of HFA-flunisolide in peripheral and central airways of patients with asthma.²⁵ In parallel, we aimed to investigate features of remodeling in central and peripheral airways of patients with mild to moderate asthma, sampled by endobronchial and transbronchial biopsy, respectively, and to examine the effect of the ICS HFA-flunisolide on these features.

METHODS

Study design

A single-center, open-label study was conducted in adult patients with asthma to evaluate the effect of inhaled small-particle corticosteroids (HFA-flunisolide) on peripheral lung inflammation and remodeling. The study was performed in full compliance with Food and Drug Administration Guidelines for Good Clinical Practice and was approved by the institutional review board. Patients who met the inclusion and exclusion criteria had a baseline bronchoscopy, during which transbronchial and endobronchial lung biopsy specimens were obtained. Patients were then treated for 6 weeks with HFA-flunisolide (340 µg [4 puffs] twice daily), and airway biopsy specimens were again taken. The effect of HFA-flunisolide on lung function (FEV₁, forced vital capacity, and forced expiratory flow at 25% to 75% of vital capacity [FEF₂₅₋₇₅]) was assessed by spirometry from visit 2, before treatment, to visit 4, after treatment (Table I and II) and was previously reported.²⁵ The effect of HFA-flunisolide on lung collagen deposition, smooth muscle mass, and TGF-β⁺ cells was measured by immunocytochemistry.

Subjects

A total of 12 patients with asthma (6 men and 6 women) were included in the study. The mean age was 33 years (22-41 years). Patients were eligible for participation in the study if they were 18 to 50 years of age, had a baseline (visit 1, recruitment) FEV₁ between 60% and 85% of the predicted value (mean of 75%, ranging from 61% to 85%) after withholding short-acting β-agonist usage for at least 6 hours, had a ≥12% increase in FEV₁ after 2 to 4 puffs of albuterol or a PD₂₀ to methacholine of <8 mg/mL, used albuterol at least 3 times per week with a mean daily use of 8 puffs per day,

had a total peripheral blood eosinophil count of >200 cells/dL, and had not used any steroids within the preceding 30 days.

Exclusion criteria were pulmonary disease other than asthma, exacerbation of asthma within 6 weeks before enrollment, use of an ICS or leukotriene modifier within 30 days of enrollment or any other steroid use within 6 weeks before enrollment, history of drug or alcohol abuse, and any significant abnormality on physical examination or laboratory evaluation.

Spirometry

Spirometry was performed after withholding albuterol for at least 6 hours, in a manner consistent with the American Thoracic Society (ATS) guidelines at visit 1, 2, and 4. At least 3 acceptable forced expiratory maneuvers were required of each patient. The FEV₁ from the best effort, as defined by the ATS, was used. All spirometry equipment was ATS-approved and calibrated on each day of use. Percent predicted values were calculated by means of the Crapo formula.

Tissue sampling and preparation

Transbronchial and endobronchial biopsy specimens were obtained from subjects before and after treatment. Under fluoroscopic guidance, a transbronchial biopsy forceps was passed through the bronchoscope to the periphery of the lung. A total of 4 transbronchial biopsy specimens were taken from the ipsilateral lower lobe, middle lobe, and upper lobe. At least 4 biopsy specimens were taken from secondary carinae under direct vision. These were taken in the same lung in which the transbronchial biopsy specimens were taken. Half of the transbronchial and endobronchial biopsy specimens were fixed in 4% paraformaldehyde, then dehydrated in alcohol and blocked in fresh molten wax for immunocytochemistry or Van Gieson staining. The other half were kept frozen for other purposes.

Van Gieson for tissue staining

After dewaxing, slides were rehydrated, then subsequently treated with celestine blue-hemalum, acid alcohol, and van Gieson dye. Slides were then dehydrated, cleared in xylene, and mounted.

Immunocytochemistry

To detect collagen III, α-smooth muscle actin, and TGF-β expression, immunocytochemistry was performed with the use of a peroxidase development on paraffin embedded section. First, slides were deparaffinized in xylene and in decreasing concentrations of alcohol, permeabilized with Triton 0.2%, and incubated with 5% peroxide. After blocking with Universal blocking solution for 30 minutes (Dako, Carpinteria, Calif), slides were incubated overnight with the following primary antibodies: specific mouse antihuman mAbs against collagen III (Medicorp, Montreal, Quebec, Canada), α-smooth muscle actin (Novocastra Laboratories Ltd, Newcastle, United Kingdom), and TGF-β (Santa Cruz Biotechnologies, Santa Cruz, Calif). After subsequent incubation with biotinylated secondary rabbit antimouse antibody (Dako) and with ABC complex (Dako), slides were stained with diaminobenzidine-chromogen (Dako) and counterstained with hematoxylin.

Quantification

All slides were read by an observer blind to group. Areas occupied by collagen, collagen III, and smooth muscle actin in muscle bundles as well as total biopsy area were measured. Positive signals appeared brown under light microscopy. The areas of interest were measured by using image analysis (Image Pro-Plus software; Media Cybernetics, Silver Springs, Md) and are presented as the percentage of stained area of total biopsy area. TGF-β immunoreactive cells were expressed as number of cells per square millimeter of submucosa.

TABLE I. FEV₁ before and after bronchodilator and HFA-flunisolide*

	FEV ₁ L pre-BD	FEV ₁ pre-BD % predicted	FEV ₁ L post-BD	FEV ₁ post-BD % predicted	% Increase in FEV ₁ post-BD
Pre-ICS	3.02 (1.7-4.17)	79 (52-94)	3.38 (2.42-4.43)	88 (74-102)	12.8 (1.2-15.4)
Post-ICS	3.2 (2.21-4.1)	84 (68-95)	3.42 (2.37-4.36)	89 (72-103)	6.5 (1.9-13.3)
% Increase in FEV ₁ post-ICS	7 (−3.1-30)				

BD, Bronchodilator.

*The mean values and the ranges for the groups are shown.

TABLE II. Mean of FEF₂₅₋₇₅ before and after bronchodilator and HFA-flunisolide*

	FEF ₂₅₋₇₅ L/s pre-BD	FEF ₂₅₋₇₅ % predicted	FEF ₂₅₋₇₅ L/s post-BD	FEF ₂₅₋₇₅ % predicted	% Increase in FEF ₂₅₋₇₅ post-BD
Pre-ICS	2.38 (0.72-4.8)	57 (20-104)	2.95 (1.44-5.81)	70 (40-126)	28 (0.1-100)
Post-ICS	2.71 (1.15-5.01)	65 (32-117)	3.11 (1.33-5.39)	74 (37-117)	14 (−2.2-33)
% Increase in FEF ₂₅₋₇₅ post-ICS	20 (−10-63)				

BD, Bronchodilator.

*The mean values and the ranges for the groups are shown.

Immunocytochemistry was performed with isotype antibodies as negative control.

Statistics

Eight biopsies were available for each subject: central airways, 2 pre-ICS and 2 post-ICS; peripheral airways, 2 pre-ICS and 2 post-ICS. On assessing biopsy quality, the mean of 2 observations was used to compare before and after outcomes. By combining the 2 biopsies, peripheral airways were detected in 75% of the subjects, and smooth muscle bundles were observed in 50% of the peripheral and central airway samples. A complete set of slides (peripheral and central airways, and before and after HFA-flunisolide) was available for at least 5 patients in each measured parameter. Our data were normally distributed, and a parametric test was used to detect differences. A paired, 1-sample *t* test was used to test the null hypothesis that there was no difference in each remodeling feature between central and peripheral airways before and after 6 weeks of HFA-flunisolide treatment. This test was against the alternative hypothesis that there was a difference in the extent of remodeling, or in the treatment effect. The power was calculated by using the NCSS-PASS program (NCSS Statistical Software, Kaysville, Utah) with a paired 1-sample *t* test. The coefficient and significance of correlation were calculated between 2 paired data sets by Spearman rank correlation formula and *t* test.

RESULTS

Collagen deposition in peripheral and central airways

At baseline, there was a significant deposition of total collagen and collagen III in both peripheral and central airways. The percentage of the airway wall occupied by collagen as identified by Van Gieson stain for total collagen was significantly higher in central airways than in peripheral airways (54.5% ± 5.6% vs 37.7% ± 6.9%; *P* = .04; Fig 1, A). However, there were no significant differences between the percentage of the airway stained for collagen III in central airways compared with peripheral airways (central, 21.6% ± 3.8%; peripheral, 20.5% ± 5.6%; *P* = .88; Fig 1, B).

Smooth muscle area in peripheral and central airways

At baseline, the percentages of the airway wall area that stained with α-smooth muscle actin in central and peripheral airways were 18.6% ± 4.9% and 13.4% ± 2.7%, respectively (Fig 1, C). These differences were not significant (*P* = .94).

Correlation between airflow obstruction and remodeling features

No correlations were observed among total collagen, collagen III, or α-smooth muscle actin areas in either peripheral or central airways and FEV₁, FEV₁/forced vital capacity, and FEF₂₅₋₇₅ (data not shown).

Effect of HFA-flunisolide on lung function parameters

Before starting the treatment, the mean FEV₁ (3.02 L) was 79% of the predicted value, and the mean FEF₂₅₋₇₅ (2.38 L) was 57% of the predicted value (Tables I and II). After 6 weeks of HFA-flunisolide treatment, FEV₁ and FEF₂₅₋₇₅ improved by 7% (*P* = .012) and 20% (*P* = .034), respectively. The reversibility of FEV₁ and FEF₂₅₋₇₅ postbronchodilator was decreased by 50% after HFA-flunisolide treatment (Table II).

HFA-flunisolide effect on remodeling features in central and peripheral airways

No changes in total collagen (central, 54.5% ± 5.6% vs 64.4% ± 7.1%, *P* = .14; peripheral, 37.7% ± 6.9% vs 32.8% ± 8.0%, *P* = .65) and collagen III (central, 21.6% ± 3.8% vs 26.8% ± 5.1%, *P* = .2; peripheral, 20.5% ± 5.6% vs 13.9% ± 4.1%, *P* = .41) were observed after HFA-flunisolide treatment in both categories of airways (Fig 2, A and B). The HFA-flunisolide treatment also had no effect on smooth muscle area in the central airways (18.6% ± 4.9% vs 27% ± 9.5%; *P* = .48; Fig 2, C).

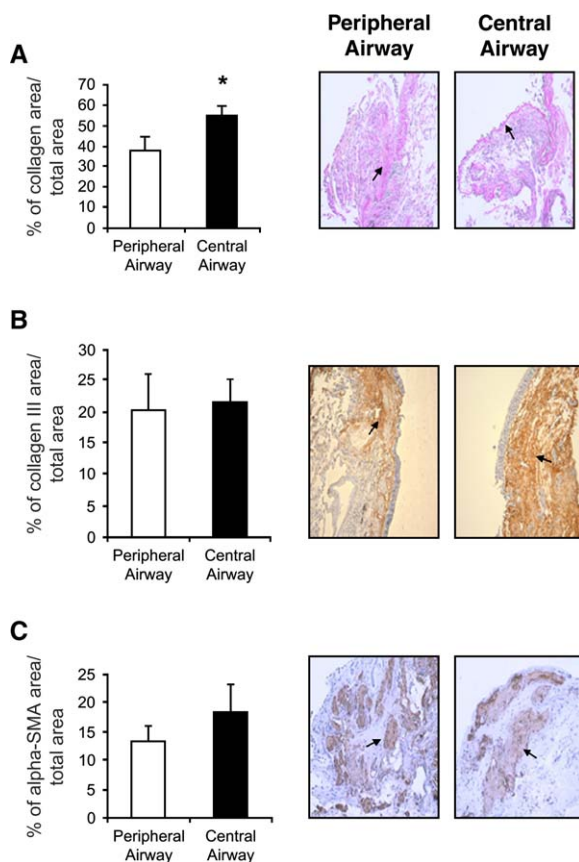


FIG 1. Remodeling features in peripheral and central airways. **A**, Total collagen deposition in peripheral and central airways; $P = .04$. **B**, Immunostaining for collagen III in peripheral and central airways; $P = .88$. **C**, Immunostaining for α -smooth muscle actin (SMA) in peripheral and central airways; $P = .94$. Representative pictures with arrows showing positive staining for Van Gieson staining total collagen (A), collagen III (B), and α -smooth muscle actin (C) in peripheral and central airways.

However, the 6-week course of HFA-flunisolide significantly decreased the area of staining for α -smooth muscle actin in the peripheral airways of our individuals with asthma ($13.4\% \pm 2.7\%$ to $4.6\% \pm 1.2\%$; $P = .01$; Fig 2, C). Despite the small sample size, the power to detect the difference was 92%. The percentage of change in baseline FEF₂₅₋₇₅ from pretreatment to posttreatment with HFA-flunisolide was negatively correlated with the change in smooth muscle mass in peripheral airways in the 5 patients for whom both physiological and biopsy data were available ($r_s = -1.00$; $P = .00$; Fig 3), representing an improvement in the static state of small airway bronchoconstriction. In other words, the more smooth muscle mass is decreased, the greater the increase in baseline flow rates.

TGF- β^+ cells in peripheral and central airways and HFA-flunisolide effect

The baseline number of TGF- β^+ cells in the airway submucosa was not significantly different between peripheral and central airways (3.6 ± 0.7 vs 6.6 ± 1.6 ; $P = .09$;

Fig 4). HFA-flunisolide treatment had no significant effect on the number of TGF- β^+ cells in both airway sizes (central, 6.6 ± 1.6 vs 7.1 ± 1.5 , $P = .81$; peripheral, 3.6 ± 0.7 vs 1.6 ± 0.96 , $P = .13$; Fig 4). The number of TGF- β^+ cells in the submucosa correlated positively with the total collagen deposition in central airways ($r_s = 0.873$; $P = .01$) but not in peripheral airways.

DISCUSSION

The current study describes the extent of collagen deposition and the smooth muscle mass in asthmatic peripheral airways by using transbronchial biopsies and addresses the effect of treatment with HFA-flunisolide on them. We observed structural similarities between central and peripheral airways. Both categories of airway had comparable amounts of collagen III deposition and α -smooth muscle actin. However, total collagen deposition was significantly higher in central airways compared with peripheral airways. From our study on airway inflammation and lung function,²⁵ we can affirm that the HFA-flunisolide treatment is effective in treating inflammation in both peripheral and central airways in this group of patients with mild to moderate asthma. We show in the current study that HFA-flunisolide is ineffective in reducing collagen deposition and TGF- β expression but has the ability to reduce the smooth muscle mass in peripheral airways. The functional significance of this finding is suggested by the correlation between the smooth muscle actin–positive area after HFA-flunisolide treatment and the improvement in FEF₂₅₋₇₅.

Compared with chlorofluorocarbon formulated flunisolide, HFA-flunisolide formulation has a higher airway deposition and reaches the more distal airways.²⁴ Accordingly, we used the latter to study the potential antiremodeling effect of ICS on peripheral airways. Furthermore, with regard to inflammation, we have previously demonstrated the effectiveness of HFA-flunisolide in both peripheral and central airways.²⁵ We used only HFA-flunisolide in this study and therefore cannot comment on whether other ICS formulations have similar properties. However, it would have been of interest to compare chlorofluorocarbon and HFA-ICS formulations. Unfortunately, we have neither a control group of normal subjects nor placebo-treated patients. However, our study design allowed us to compare central and peripheral airway remodeling features in the same individuals with asthma, and our observations can be compared with historical data. Chu et al¹⁰ reported that total collagen represented 39.6% of total area of endobronchial biopsy from patients with mild asthma and that 39.9% of total collagen was collagen III. In our study, we found total collagen deposition to be 54.5% of total biopsy area in central airways, whereas collagen III occupied 39% of the airway wall, which is in agreement with Chu et al.¹⁰ The discrepancy in the total collagen area might result from the differences in staining methods. Chu et al¹⁰ used Sirius Red staining, in contrast with the Van Gieson staining

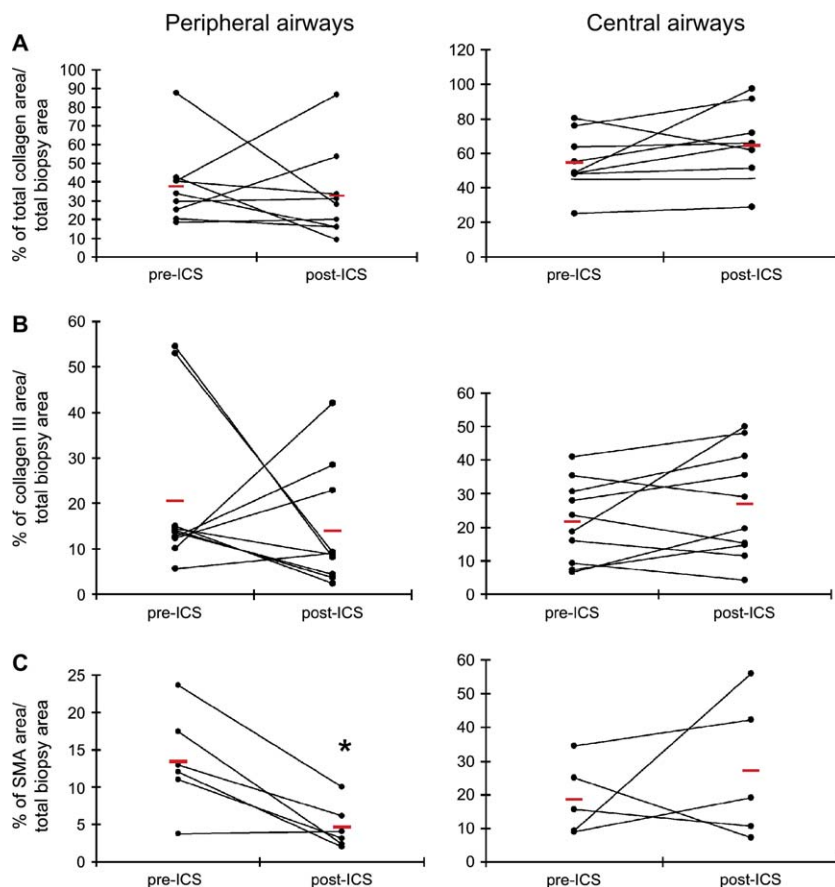


FIG 2. Effect of HFA-flunisolide on remodeling features. **A**, Total collagen deposition before and after 6 weeks of HFA-flunisolide treatment in peripheral ($P = .65$) and central ($P = .14$) airways. **B**, Collagen III immunostaining before and after 6 weeks of HFA-flunisolide treatment in peripheral ($P = .41$) and central ($P = .20$) airways. **C**, α -Smooth muscle actin (SMA) area before and after 6 weeks of HFA-ICS treatment in peripheral ($P = .01$) and central ($P = .48$) airways.

method we used. We observed a higher deposition of total collagen in central compared with peripheral airways (54.5% vs 37.7%; $P = .04$). However, collagen III deposition was similar in central and peripheral airways (21.6% vs 20.5%). James et al¹² have reported a positive correlation between central airway reticular basement membrane thickness and the remodeling in peripheral cartilaginous airways but not in membranous airways. In their study, the extent of remodeling or collagen deposition in the peripheral airways was not mentioned. We observed no correlation between total submucosal collagen and airflow obstruction in peripheral and central airways. This is in concordance with the finding of Chu et al,¹⁰ in which neither the total submucosal collagen deposition nor the collagen I and III deposition was correlated with disease severity or lung function. Other studies have shown such a correlation, but the patient population was different.^{1,11,26}

Increase in smooth muscle mass is an important feature in asthma, potentially leading to airflow obstruction and hyperresponsiveness.^{27,28} Our study showed that 18.6% of the total biopsy area was occupied by smooth muscle in central airways and 13.4% in peripheral airways with

no significant difference between airway sizes. Our measurement of smooth muscle area as a percentage of the biopsy area in central airways is similar to that reported by Benayoun et al,²⁸ 17.5%, in a group of patients with mild to moderate asthma. It is reasonable to hypothesize that the smooth muscle mass observed in peripheral airways is increased. The smooth muscle mass is quantified by measuring the area of muscle bundles that are positive for α -smooth muscle actin. This protein is present in smooth muscle cells and in myofibroblasts. It is possible that myofibroblasts are included in our measurements. These cells are reported to contribute to the increased smooth muscle mass in fatal asthma.²⁹

Taking into consideration the previous reports and the current data presented in this article, we suggest that remodeling, including increased collagen deposition and smooth muscle mass, is present in peripheral airways of patients with mild to moderate asthma. To our knowledge, this is the first study that specifically measured collagen III and smooth muscle actin in peripheral airways.

The number of TGF- β^+ cells in the submucosa was similar in both airway sizes. We did not phenotype the TGF- β^+ cells. However, previous studies^{11,30} have shown

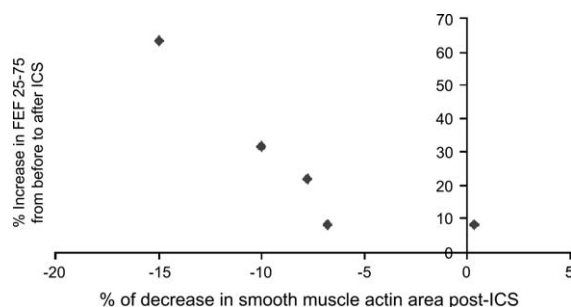


FIG 3. Correlation between the change in smooth muscle area and prebronchodilator FEF₂₅₋₇₅ improvement in the peripheral airways from before to after HFA-flunisolide treatment ($r_s = -1.00$; $P = .00$).

the airway TGF- β^+ cells to be eosinophils and fibroblasts. TGF- β increases expression of extracellular matrix proteins, including collagen I and III, fibronectin, and proteoglycan,³¹⁻³³ and decreases matrix-degrading protease levels in *in vitro* models.³⁴ In our study, the number of TGF- β^+ cells positively correlated with the total collagen deposition in central airways ($r_s = 0.873$; $P = .01$), supporting the profibrotic properties of TGF- β . However, this correlation was not seen in peripheral airways. The modest size of our sample, resulting in a power of only 67% of finding a difference, could explain the absence of correlation in the peripheral airways between collagen and TGF- β expression.

Many studies have examined the putative antiremodeling effect of corticosteroids, and most of them concluded that corticosteroids are ineffective in reducing collagen deposition.³⁵⁻³⁷ Some studies have shown a slight decrease of collagen with ICS in asthmatic airways.^{22,38-41} These studies used high doses of ICS and a long treatment course. In the current study, a 6-week course of HFA-flunisolide had no significant effect on total collagen, collagen III deposition, and TGF- β expression, which finding is consistent with the majority of published reports. The inability of corticosteroids to reduce collagen deposition is observed in both peripheral and central airways. The resistance of TGF- β in response to HFA-ICS is in agreement with a study of patients with severe asthma treated with oral corticosteroids²³ and offers a potential explanation for the failure of steroids to modulate collagen deposition.

We were surprised to find an effect of ICS on smooth muscle mass in peripheral airways. After the 6-week course of HFA-flunisolide, we observed a significant decrease in smooth muscle actin staining in peripheral airways but not central airways. *In vitro* corticosteroids have been shown to reduce smooth muscle cell proliferation.⁴²⁻⁴⁴ However, a recent study on asthmatic-derived airway smooth muscle cells reported the absence of anti-proliferative effect of corticosteroids, attributed to the lack of CCAAT/enhancer binding protein alpha (C/EBP α).⁴⁵ It is difficult to extrapolate *in vitro* studies to our *in vivo* model. It will be of interest to see if C/EBP α is present in our asthmatic airways. Nevertheless, a decreased rate of proliferation might explain the HFA-

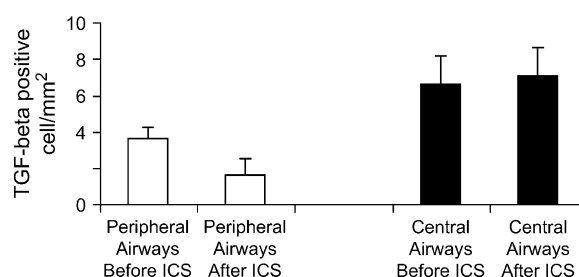


FIG 4. Number of TGF- β^+ cells in the submucosa before and after 6 weeks of HFA-flunisolide treatment in peripheral ($P = .13$) and central ($P = .81$) airways.

flunisolide effect on smooth muscle in asthmatic peripheral airways.

Peripheral airways seem to be more sensitive to HFA-flunisolide than central airways. It is possible that HFA-flunisolide is more accessible to smooth muscle bundles in thinner peripheral airways. In spite of the small size of our group, we were able to observe a correlation between change in lung function and smooth muscle mass reduction in peripheral airways. The presence of this correlation reinforces the significance of smooth muscle in determining small airway physiology. FEF₂₅₋₇₅ is a simple measure thought to reflect flow in small airways with limited interpretation related to its nonnormally distributed predicted value.⁴⁶ Unfortunately, we did not have other measures of peripheral airway physiology. Nonetheless, a negative correlation was observed between peripheral airway smooth muscle area and flow rates after HFA-flunisolide treatment ($r_s = -1.00$; $P = .00$), suggesting the beneficial effect of the reduced smooth muscle mass. Furthermore, a 50% decrease in postbronchodilator reversibility in FEF₂₅₋₇₅ was observed after the HFA-flunisolide treatment, reflecting a decrease in lower airway reactivity. Hence, HFA-flunisolide could be considered effective in reversing disease at the peripheral airway level.

In summary, we have demonstrated that airway remodeling in peripheral airways in patients with mild to moderate asthma is comparable with that in the central airways. We also confirm the inability of ICS to modulate collagen deposition and TGF- β expression. It is interesting to show that HFA-flunisolide was associated with a significant decrease in the expression of smooth muscle actin in peripheral airways, which was correlated with an improvement in airway function.

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