

Airway Smooth Muscle Hypertrophy and Hyperplasia in Asthma

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Rationale: Increased thickness of the airway smooth muscle (ASM) layer in asthma may result from hyperplasia or hypertrophy of muscle cells or increased extracellular matrix (ECM).

Objectives: To relate ASM hypertrophy, ASM hyperplasia, and deposition of ECM to the severity and duration of asthma.

Methods: Airways from control subjects ($n = 51$) and from cases of nonfatal ($n = 49$) and fatal ($n = 55$) asthma were examined postmortem. Mean ASM cell volume (V_C), the number of ASM cells per length of airway (N_L), and the volume fraction of extracellular matrix (f_{ECM}) within the ASM layer were estimated. Comparisons between subject groups were made on the basis of general linear regression models. **Measurements and Main Results:** Mean V_C was increased in the large airways of cases of nonfatal asthma ($P = 0.015$) and fatal asthma ($P < 0.001$) compared with control subjects. N_L was similar in nonfatal cases and control subjects but increased in large ($P < 0.001$), medium ($P < 0.001$), and small ($P = 0.034$) airways of cases of fatal asthma compared with control subjects and with nonfatal cases (large and medium airways, $P \leq 0.003$). The f_{ECM} was similar in cases of asthma and control subjects. Duration of asthma was associated with a small increase in N_L .

Conclusions: Hypertrophy of ASM cells occurs in the large airways in both nonfatal and fatal cases of asthma, but hyperplasia of ASM cells is present in the large and small airways in fatal asthma cases only. Both are associated with an absolute increase in ECM. Duration of asthma has little or no effect on ASM hypertrophy or hyperplasia or f_{ECM} .

Keywords: nonfatal asthma; fatal asthma; extracellular matrix; asthma duration

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

This study shows that both hypertrophy and hyperplasia contribute to the increased thickness of the layer of airway smooth muscle (ASM) in asthma. Hypertrophy occurs in both nonfatal and fatal cases of asthma, but hyperplasia is present predominantly in cases of fatal asthma and involves large and small airways.

What This Study Adds to the Field

The findings suggest that different forms of remodeling of ASM may account for differences in the severity of asthma and that these changes are present early in the natural history of asthma.

Hypertrophy and hyperplasia of airway smooth muscle (ASM) cells are likely to increase the ability of the ASM to shorten, and changes in the volume and the composition of the extracellular matrix (ECM) may alter the loads that oppose shortening of smooth muscle (1, 2). The thickness of the ASM layer around the airways is increased in individuals with asthma, compared with nonasthmatic control subjects (3–11), and hyperplasia (12–14) and hypertrophy (12, 13) have been reported to contribute to the increased ASM thickness. Distinct pathways may lead to hypertrophy or hyperplasia (15) or to increased deposition of ECM (16). How these processes are related to airway size and to the duration and severity of asthma will influence how appropriate treatment and preventive strategies for remodeling of the ASM layer in asthma are developed.

The ASM layer is made up of smooth muscle, matrix, inflammatory cells, mast cells, blood vessels and, in tissue sections, space between tissue structures because of fixation artifact. These elements may overlap and contribute to the estimated volume of ASM cells. Previous studies have used tissues only from the large airways (6, 13, 14, 17) or from a small number of clinically severe and fatal cases of asthma (12). We have examined hypertrophy and hyperplasia in large and small airways in a large number of cases of asthma of varying clinical severity and used thin sections to estimate the volume fraction of ASM cells and ECM within the smooth muscle layer. The effects of subject variables such as age and duration and severity of asthma on remodeling of the ASM were also examined.

METHODS

Approval for this study was obtained from the institutional ethics committees of the participating centers and from the Sir Charles Gairdner

Hospital Human Research Ethics Committee. More details of the methods are available in the online supplement.

Subjects

Tissues were derived from six postmortem studies of asthma based in Perth (3), Melbourne (18), Auckland (19), Calgary (20, 21), Sydney (22), and São Paulo (23) (*see* Table E1 in the online supplement). Subjects were categorized as control (no history of asthma or other lung disease), nonfatal asthma (nonrespiratory cause of death, but a history of asthma), or fatal asthma (died from asthma). Information regarding the history of asthma was sought from next-of-kin, hospital files, coroner files, and the subjects' usual medical practitioners. When possible, asthma cases were categorized as clinically mild, moderate, or severe (24). Duration of asthma was defined as the age at death less the stated age of onset of asthma. Smoking status at the time of death was categorized as current, ex-smoker, or never-smoker but as ever-smoker or never-smoker for analyses.

Tissue Preparation

Airways were sampled according to a systematic design (3, 21, 22) or at the discretion of the pathologist (18, 19, 23), fixed, and embedded in paraffin (Table E2). From each airway, two transverse sections were cut: a thin section (0.5 μm , Masson trichrome technique) for estimation of tissue volume fractions within the ASM layer and a thick section (30 μm , hematoxylin) to estimate the numerical density of ASM cells.

Airway Measurements

Volume fractions of airway smooth muscle (f_{ASM}), extracellular matrix (f_{ECM}), and "Other" (f_{Other}) within the ASM layer were estimated on the basis of point counts (Figure 1A). Airway size, defined by the reticular basement membrane perimeter (Pbm) (25) and the total area of the smooth muscle layer (ASM_{area}), were measured by planimetry on thick sections. The "optical disector" approach (26) was used to count ASM cell nuclei (Figure 1B) and to estimate cell numerical density (N_V). Because thick transverse sections were scanned in the dimension of airway length, the total number of cells per millimeter length of airway (N_L) was calculated as follows: $N_L = N_V (\text{cells}/\text{mm}^3) \times \text{ASM}_{\text{area}} (\text{mm}^2) \times 1$. Mean ASM cell volume (V_C) was calculated as the inverse of N_V , corrected for f_{ASM} : $V_C = 1/[\Sigma Q/(a \cdot h \cdot \Sigma P \times f_{\text{ASM}})]$, where Q is the number of cells counted per disector, a and h are the area and height of the disector, respectively, and P is the number of disectors counted per section.

Data Analysis

Analyses for airway sizes combined, or for airways grouped by size: small (Pbm < 4 mm), medium (Pbm = 4–10 mm) or large (Pbm > 10 mm), were undertaken with SPSS version 17.0 (SPSS Inc., Chicago, IL). The main outcomes of interest were $\text{ASM}_{\text{area}}/\text{Pbm}$, N_L , V_C (corrected), and the volume fractions f_{ASM} and f_{ECM} . The effects of center, subject group (control, nonfatal or fatal asthma), age, sex, airway size, smoking, height, and weight on outcomes, unadjusted for other variables, were examined by analysis of variance and post hoc tests as appropriate or by Pearson correlation and, to adjust for other variables, using multiple linear regression models ("general linear models"). Because there were no significant differences between the Australian

(Sydney, Melbourne, and Perth) centers, these were combined for subsequent analyses. A random effects model was also used for all airways with subject as a random effect and found essentially the same relationships of variables to outcomes. For clarity, only the results of the general linear models are presented.

RESULTS

Subject characteristics are shown in Table 1. There were 51 control subjects, 49 cases of nonfatal asthma, and 55 cases of fatal asthma. Subject groups were similar, apart from weight, which was greater in the nonfatal asthma group. The cases of fatal asthma were of greater clinical severity than the cases of nonfatal asthma (chi-square = 7.186, $P = 0.007$), but reported ages of onset and duration of asthma were similar. A total of 635 blocks were examined with a mean of 4.6 blocks per subject, with 1.1 airways per block, yielding 5 ± 2 (mean $\pm$ SD) airways per subject (*see* also Table E3). Unadjusted airway dimensions and ASM parameters for all airways and for airway size groups by subject group are shown in the online supplement (Table E4). The results from the general linear model for age, sex, subject group, center, and Pbm on ASM parameters in control subjects and subjects with asthma are shown in Table 2, Figure 2 and in the online supplement (Table E5).

Components of the ASM Layer

$\text{ASM}_{\text{area}}/\text{Pbm}$ was related significantly to subject group ($P = 0.002$), airway size ($P < 0.001$), and center ($P = 0.034$), with an interaction between center and airway size ($P = 0.002$) when airway sizes were combined. There was no significant effect of sex or age. $\text{ASM}_{\text{area}}/\text{Pbm}$ was increased in cases of fatal ($P < 0.001$) and nonfatal asthma ($P = 0.006$) compared with control subjects, and in fatal cases of asthma compared with nonfatal cases of asthma ($P < 0.001$) (Figure 2A and Table 2). These differences were also observed when examined by airway size. There were significant effects on f_{ASM} for subject group ($P = 0.023$), but not for sex, center, age, or airway size, when airway sizes were combined. Compared with control subjects, f_{ASM} was increased in cases of fatal asthma ($P = 0.008$), but not in cases of nonfatal asthma ($P = 0.492$) (Figure 2B and Table 2). Compared with cases of nonfatal asthma, f_{ASM} was increased in cases of fatal asthma ($P = 0.034$) and this effect was confined to the large airways ($P = 0.018$). For f_{ECM} , when airway sizes were combined, there was a significant effect for subject group ($P = 0.025$), but not for sex, center, age, or airway size. There were no significant differences between individual subject groups (Figure 2C and Table 2). There were no significant effects on other components (f_{Other}) within the layer of ASM for all airways combined (Table 2).

Numerical Density and Mean Volume of ASM Cells

When airway sizes were combined, there were significant effects on N_V for subject group ($P = 0.003$) and center ($P = 0.031$), but

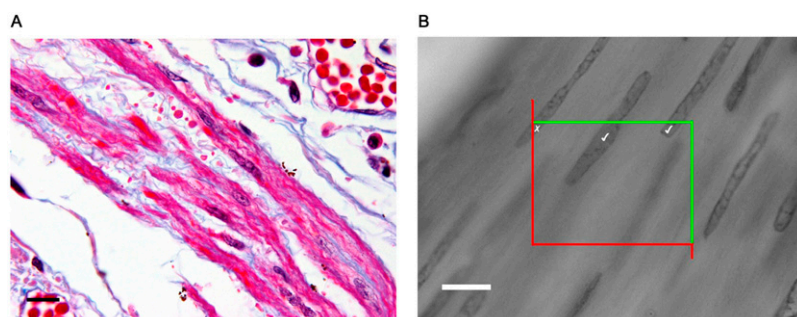


Figure 1. (A) Thin section (0.5 μm) of airway smooth muscle layer stained by the Masson trichrome technique (original magnification, $\times 1,000$). Airway smooth muscle stains red; extracellular matrix, blue; and cell nuclei, dark purple. Spaces between the airway smooth muscle cells (categorized as "Other": blood vessels, space, inflammatory cells) are visible on thin sections. (B) Thick section (30 μm) of airway smooth muscle layer stained with hematoxylin (original magnification, $\times 1,000$) to count airway smooth muscle cell nuclei, using the optical disector. Scale bars: 10 μm . A tick symbol is a counted nucleus; an x symbol is an excluded nucleus.

TABLE 1. SUBJECT CHARACTERISTICS

	Control (n = 51)	Nonfatal Asthma (n = 49)	Fatal Asthma (n = 55)
Sex, M/F	34/17	27/22	31/24
Age, yr: median (IQR)	33 (18–43)	29 (21–41)	35 (19–48)
Height, * cm: mean $\pm$ SD	172 $\pm$ 13	169 $\pm$ 13	170 $\pm$ 11
Weight, * kg: median (IQR)	69 [†] (59–81)	85 (66–105)	71 [†] (59–81)
Smoking, * n			
Current	17	15	11
Never smoked	12	19	17
Ex-smoker	3	4	4
Data unavailable	19	11	23
Age at onset of asthma, * yr: median (IQR)	—	12 (3–24)	7 (2–24)
Duration of asthma, * yr: median (IQR)	—	16 (10–21)	18 (11–25)
Asthma severity, * n	—		
Mild–moderate	—	21	7
Severe	—	13	19 [†]

Definition of abbreviations: F = female; IQR = interquartile range (25% percentile–75% percentile); M = male.

*Data not available for all subjects.

[†] $P < 0.05$ versus nonfatal asthma.

not airway size, sex, or age. Compared with control subjects, N_V was decreased in cases of fatal asthma ($P < 0.001$) and in cases of nonfatal asthma ($P = 0.002$) (Figure 2D and Table 2). There were significant effects on V_C for subject group ($P = 0.003$), sex ($P = 0.025$), and center ($P < 0.001$), but not for airway size or age when airway sizes were combined. V_C was increased in males compared with females ($P = 0.008$). Compared with control subjects, V_C was increased in cases of fatal asthma ($P = 0.005$) and nonfatal asthma ($P = 0.014$), which were similar to each other (Figure 2E and Table 2). The increase in V_C was in the large airways in both fatal asthma ($P < 0.001$) and nonfatal asthma ($P = 0.015$).

Number of Cells per Unit Length of Airway

There were significant effects on N_L for subject group ($P = 0.025$), airway size ($P < 0.001$), and center ($P = 0.013$), but not for sex or age when airway sizes were combined. There was a significant interaction between center and airway size ($P < 0.001$). Compared with control subjects, N_L was increased in cases of fatal asthma ($P < 0.001$), but not in cases of nonfatal asthma ($P = 0.086$) (Figure 2F and Table 2). N_L was increased in cases of fatal asthma in all airway sizes. N_L was also increased in cases of fatal asthma compared with cases of nonfatal asthma for airway sizes combined ($P < 0.001$) and in medium ($P < 0.001$) and large ($P = 0.003$) airways.

Effects of Age and Duration of Asthma

For the combined asthma groups (online supplement, Table E6), there were no significant effects of age on ASM cell number or volume and only a small but significant effect on f_{Other} ($P = 0.039$) when all airways were combined. The duration of asthma had a small positive effect, independent of age, on $\text{ASM}_{\text{area}}/\text{Pbm}$ ($P = 0.012$) and N_L ($P = 0.042$; Figure 3A) in large airways. Within asthma groups (data not shown), there was a significant effect of age on $\text{ASM}_{\text{area}}/\text{Pbm}$ in the large airways of cases of fatal asthma ($P = 0.05$) and of duration of asthma on $\text{ASM}_{\text{area}}/\text{Pbm}$ in the large airways of cases of nonfatal asthma ($P = 0.018$) but no effects of age or duration of asthma on ASM cell number (Figure 3A) or size (Figure 3B) or on tissue fractions within the ASM layer.

Other Subject Characteristics

History of smoking cigarettes, height, and body weight did not have independent effects on ASM parameters. Because of small numbers, the effects of various forms of medication could not be analyzed.

DISCUSSION

The study shows that when adjusted for study center, age, sex, and airway size, the increased thickness of the layer of ASM in asthma is due to hyperplasia of ASM cells in the large, medium, and small airways in fatal cases of asthma, and to hypertrophy of ASM cells in the large airways in cases of both fatal and nonfatal asthma. Significant hyperplasia was not observed in cases of nonfatal asthma.

In general, there were no significant changes in the volume fraction of ECM or muscle in the ASM layer, suggesting that there was a concomitant increase in the amount of tissue matrix along with the increase in ASM cell size and number. The exception was in the large airways of fatal cases of asthma, where the fraction of smooth muscle was increased and the fraction of ECM was decreased. There were no significant effects seen for age, but increased duration of asthma was associated with a small increase in ASM cell number in cases of asthma. The findings suggest that asthma (mild, moderate, or severe) is characterized by ASM cell hypertrophy in the large airways and that fatal asthma (predominantly severe) is characterized by hyperplasia of ASM cells in both the large and small airways. Because clinical data were not available for all subjects and because clinical severity was strongly associated with the asthma group (fatal or nonfatal), we could not detect an effect of clinical severity that was independent of asthma group. Both hypertrophy and hyperplasia of ASM were accompanied by an increase in ECM such that the volume fractions of muscle and matrix were generally, except for large airways in fatal asthma, similar in asthmatic and nonasthmatic airways.

A number of previous studies have examined ASM hyperplasia and hypertrophy in asthma (6, 12–14). This study has shown that hyperplasia is present in cases of clinically severe, fatal asthma. This agrees with the observations of Heard and Hossain (6) and Ebina and colleagues (12), who compared cases of fatal asthma with nonasthmatic individuals. Although there was a trend in the large airways ($P = 0.086$), we did not find a statistically significant increase in the number of ASM cells per length of airway in cases of nonfatal asthma. This agrees with the findings of Regamey and colleagues (13), who found hyperplasia in cases of moderate–severe asthma, similar to the clinical severity of asthma in our cases of fatal asthma. Our results contrast with the study by Woodruff and colleagues (14), who reported hyperplasia in cases of mild–moderate asthma. In that study, biopsies of central airways were examined whereas in the present study, the large airways included all cartilaginous airways, many of which would not normally be accessible to biopsy by bronchoscope. In addition, our cases of nonfatal asthma were from the general community, unlike the clinic patients in other studies (13, 14), and therefore likely to be less severe. Taken together, the results of all studies suggest a relation between the number of ASM cells in the central airways and the clinical severity of asthma.

We found that hypertrophy was present in the large airways of cases of both fatal and nonfatal asthma. Previous studies have observed hypertrophy in postmortem studies of whole airway sections from fatal cases of asthma (12), but not in biopsies from less severe cases of asthma (13, 14). In biopsy studies, a small sample of tissue is necessarily obtained for examination. Estimation of the mean ASM cell volume is based on the density of

TABLE 2. AIRWAY SMOOTH MUSCLE LAYER PARAMETERS IN SMALL, MEDIUM, AND LARGE AIRWAYS, AND ALL AIRWAYS COMBINED, IN SUBJECTS WITH AND WITHOUT ASTHMA

Airway Size	Control	Nonfatal Asthma	Fatal Asthma
Airway smooth muscle layer thickness (ASM _{area} /Pbm), mm			
All airways	0.025 (0.022–0.029)	0.033* (0.029–0.037)	0.079 ^{†,‡} (0.063–0.100)
<4 mm	0.017 (0.013–0.022)	0.022 [§] (0.017–0.030)	0.027* (0.022–0.033)
4–10 mm	0.019 (0.016–0.022)	0.024 [§] (0.020–0.028)	0.063 ^{†,‡} (0.052–0.077)
>10 mm	0.043 (0.029–0.062)	0.061* (0.042–0.089)	0.089 ^{†,} (0.064–0.123)
Fraction of airway smooth muscle (f _{ASM})			
All airways	0.64 (0.60–0.68)	0.65 (0.61–0.69)	0.67* [¶] (0.64–0.71)
<4 mm	0.69 (0.66–0.72)	0.69 (0.65–0.72)	0.69 (0.66–0.72)
4–10 mm	0.67 (0.64–0.70)	0.65 (0.62–0.68)	0.66 (0.64–0.69)
>10 mm	0.61 (0.57–0.65)	0.63 (0.59–0.66)	0.65 [§] (0.61–0.69)
Fraction of extracellular matrix (f _{ECM})			
All airways	0.19 (0.18–0.20)	0.18 (0.17–0.19)	0.19 (0.16–0.21)
<4 mm	0.20 (0.18–0.23)	0.20 (0.17–0.23)	0.19 (0.16–0.21)
4–10 mm	0.17 (0.16–0.18)	0.17 (0.15–0.18)	0.16 (0.14–0.18)
>10 mm	0.19 (0.17–0.22)	0.19 (0.16–0.21)	0.16 ^{§,} (0.14–0.19)
Fraction of "other" (f _{Other})			
All airways	0.15 (0.12–0.17)	0.15 (0.12–0.17)	0.15 (0.13–0.17)
<4 mm	0.12 (0.05–0.20)	0.13 (0.05–0.20)	0.14 (0.07–0.21)
4–10 mm	0.14 (0.12–0.16)	0.17 [§] (0.15–0.18)	0.18* (0.16–0.20)
>10 mm	0.20 (0.17–0.23)	0.19 (0.16–0.22)	0.19 (0.16–0.22)
Airway smooth muscle cells, numerical density (N _V), cells/mm ³ , × 10 ⁵			
All airways	2.738 (2.550–2.966)	2.333* (2.168–2.413)	2.221 [†] (2.042–2.413)
<4 mm	3.038 (2.547–3.626)	2.697 (2.255–3.229)	2.583 (2.277–2.930)
4–10 mm	2.878 (2.568–3.193)	2.452 [§] (2.210–2.724)	2.138 [†] (1.897–2.413)
>10 mm	2.380 (2.206–2.583)	2.028 [§] (1.870–2.192)	1.657 ^{†,} (1.418–1.939)
Airway smooth muscle cell volume (V _C), μm ³ , × 10 ³			
All airways	2.757 (2.550–2.962)	3.118 [§] (2.916–3.320)	3.257* (2.983–3.532)
<4 mm	2.419 (1.979–2.859)	2.695 (2.248–3.141)	2.569 (2.183–2.953)
4–10 mm	2.863 (2.416–3.310)	3.104 (2.662–3.546)	3.225 (2.833–3.616)
>10 mm	2.806 (2.322–3.292)	3.253 [§] (2.777–3.729)	3.605 [†] (3.162–4.048)
Airway smooth muscle cell number per millimeter length of airway (N _L), cells/mm, × 10 ⁵			
All airways	0.611 (0.523–0.712)	0.731 (0.629–0.845)	1.670 ^{†,‡} (1.293–2.156)
<4 mm	0.134 (0.099–0.181)	0.165 (0.122–0.224)	0.189 [§] (0.153–0.234)
4–10 mm	0.339 (0.287–0.398)	0.374 (0.321–0.439)	0.846 ^{†,‡} (0.703–1.018)
>10 mm	1.297 (0.899–1.872)	1.539 (1.060–2.235)	2.293 ^{†,} (1.660–3.165)

Airway size defined as follows: small airways, basement membrane perimeter (Pbm) < 4 mm; medium airways, Pbm = 4–10 mm; large airways, Pbm > 10 mm.

Data represent means and 95% confidence intervals adjusted for age, sex, center, and airway size (Pbm).

* $P < 0.01$ versus control.

† $P < 0.001$ versus control.

‡ $P < 0.001$ versus nonfatal asthma.

§ $P < 0.05$ versus control.

|| $P < 0.01$ versus nonfatal asthma.

¶ $P < 0.05$ versus nonfatal asthma.

ASM cells per volume of tissue. Considering that very small volumes of tissue are obtained in a bronchoscopic biopsy, the variability of the tissue fractions between biopsies will increase the chance of a type II error.

The fraction of the ASM layer that was occupied by ECM in the present study was similar in the asthma cases and control subjects (despite the increase in cell number and/or size), except in the large airways in cases of fatal asthma, where it was decreased and the volume fraction of ASM was increased. That is, the increased absolute volume of muscle within the ASM layer was matched by an increase in the absolute volume of ECM, albeit to a lesser extent in the large airways in cases of fatal asthma. The findings in cases of fatal asthma agree with those of Bai and colleagues (27), who showed that there was an increase in the area of both muscle and ECM, and an increase in the fraction of ASM in the airway wall in cases of fatal asthma compared with control nonasthmatic subjects. It has been postulated that ECM within the airway wall in asthma may act to protect the airway from excessive narrowing (1). If the mechanical properties of the ECM and its interaction with the ASM are unchanged (and this may not be the case), the observed small reduction in the

f_{ECM} in the large airways of cases of fatal asthma suggests that this mechanism is unlikely.

The numerous subjects examined in the present study were derived from six centers, but all cutting and staining of sections and all measurements were undertaken by the same investigators (J.G.E. and R.L.J.). There were differences between the centers regarding time of collection, sampling strategies, and methods of fixation. Although we have not observed effects on the relationship between airway parameters within centers (28), there were significant effects of center and it was included in the regression analyses. The effects of center persisted in the multivariate regression analysis and suggested that the thickness of the ASM layer was increased and the fraction of ASM cells was decreased mainly in the large airways in Perth cases compared with those from Calgary (more details are available in the online supplement). The cause for these differences is not clear, although it might include artifact related to fixation. There was insufficient detailed information on medication in each case to allow meaningful analysis of the effects of medication.

Other sources of potential variability include variations of parameters within and between airways within subjects and

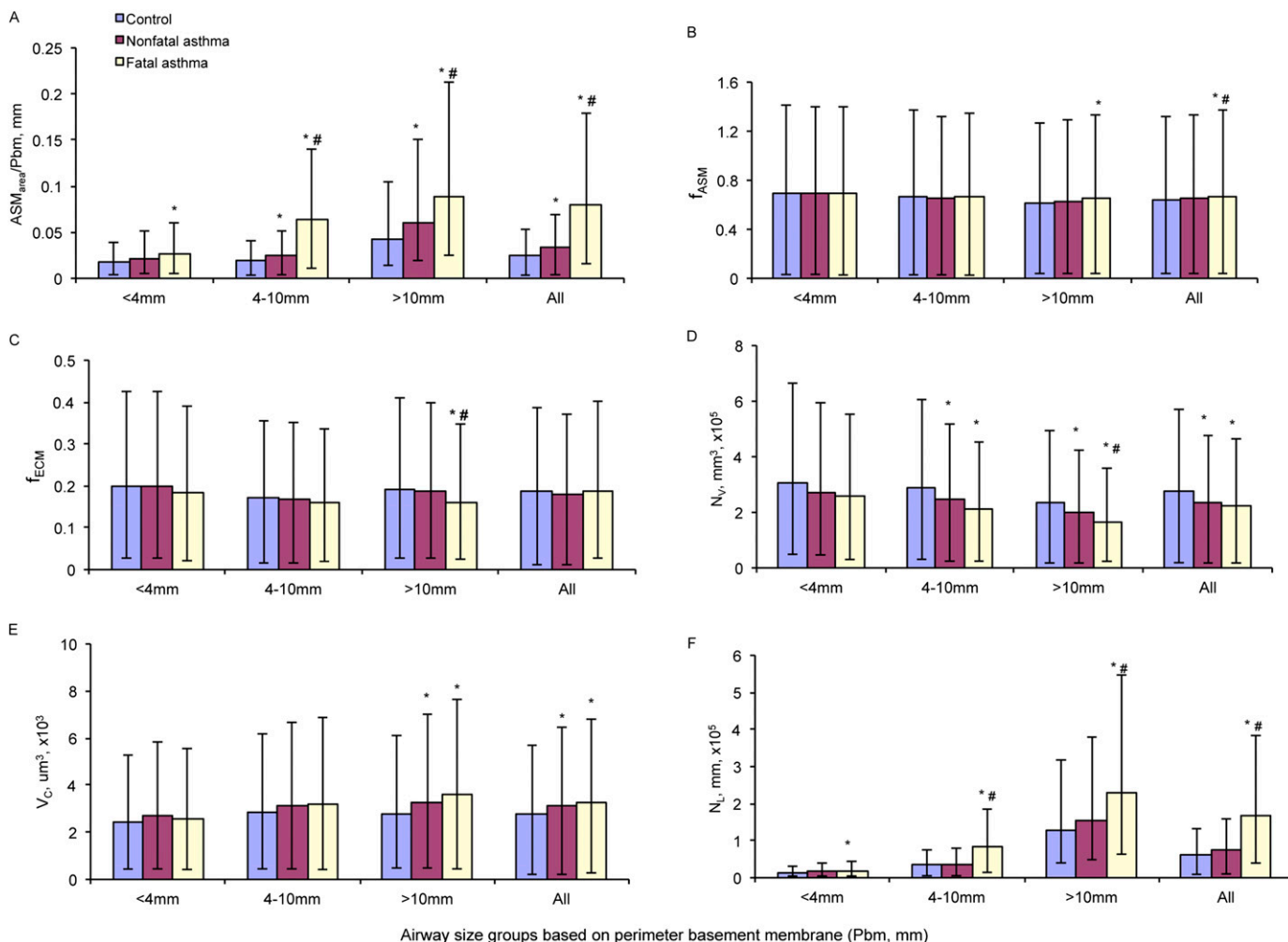


Figure 2. Airway smooth muscle (ASM) parameters (mean $\pm$ 95% confidence interval), adjusted for age, sex, study center, and airway size (based on basement membrane perimeter, Pbm). (A) Thickness of the ASM layer relative to the basement membrane length (ASM_{area}/Pbm , mm); (B) volume fraction of smooth muscle within the layer of ASM (f_{ASM}); (C) volume fraction of ECM within the layer of ASM (f_{ECM}); (D) numerical density of ASM cells (N_V , cells/mm³); (E) mean volume of ASM cells (V_C , μm^3); (F) number of ASM cells per millimeter length of airway (N_L , cells/mm). * $P < 0.05$ versus control subjects; # $P < 0.05$ versus nonfatal asthma.

uneven sampling of airways from different subjects. We have previously examined sources of variability in undertaking these stereological estimates of ASM cell number and size (R. Jones, J. Elliot, and A. James, unpublished observations). We have found that 40 disectors within each section are necessary to accurately estimate ASM cell numerical density (N_V) and that there is little

variation in N_V along airway segments, that tissue volume fractions vary less than 10% around the airway circumference or along the airway segment, but that f_{ASM} is significantly different between large and small airways. We have used methods of sampling to minimize the effect of these sources of variation. The number of airways available for each subject was not the

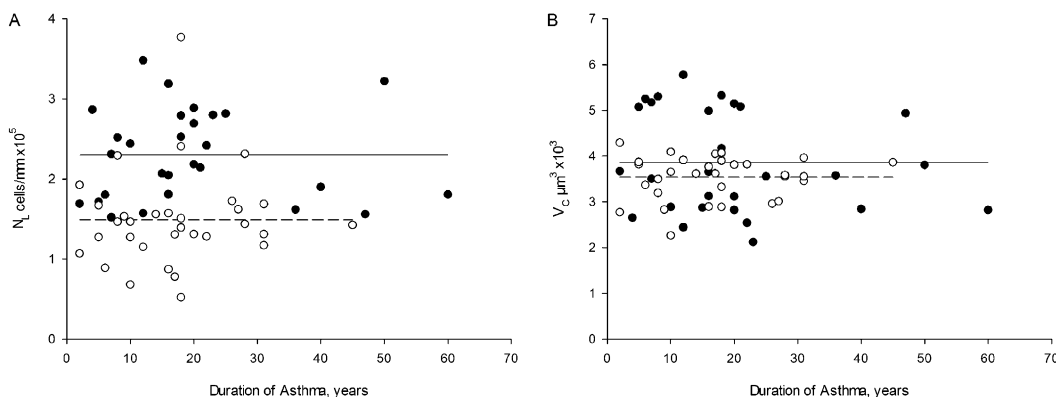


Figure 3. Duration of asthma and airway smooth muscle (ASM) cell parameters in large airways, adjusted for age, sex, center, and airway size. Open circles, nonfatal asthma; solid circles, fatal asthma. (A) The number of ASM cells per unit length of airway (N_L) in cases of nonfatal asthma (dashed line, $r = 0.082$; $P = 0.656$) and fatal asthma (solid line, $r = 0.034$, $P = 0.862$). (B) The average ASM cell volume (V_C) in cases of nonfatal asthma (dashed line, $r = 0.089$, $P = 0.626$) and fatal asthma (solid line, $r = 0.195$, $P = 0.321$).

same. Therefore, the mean value of each parameter for each airway size in each subject was used for analyses. In addition, a separate analysis was undertaken using all available airways in a random effects model with a term for subject. This produced essentially the same results.

The increased thickness of the ASM layer in asthma is generally considered a progressive response to inflammation, related usually to allergy (29) and/or epithelial injury (30). Numerous studies have shown that ASM from patients with asthma demonstrates altered secretory and growth behavior compared with ASM from subjects without asthma (31, 32). *In vitro* and animal studies show that both hypertrophy and hyperplasia can be induced in ASM cells in response to various stimuli (33–35) including inflammation (36, 37), and repeated ASM contraction may itself induce airway remodeling (38). Until recently, studies have not found increased rates of *in situ* ASM cell proliferation in patients with asthma (39, 40). However a more recent study has shown that in contrast to patients with moderate asthma, those with severe asthma had increased expression of the proliferation marker PCNA (proliferating cell nuclear antigen) in ASM cells (41). Further studies are clearly required in severe cases of asthma.

Longitudinal studies of asthma show little change in lung function (relative to control) (42–44) or asthma severity (43) over time, and structural changes, especially area of ASM, has been shown to be related to airflow obstruction in children (45). These observations suggest that the structural changes in the asthmatic airway, including the increase in thickness of the ASM layer, occur early in the natural history of asthma, particularly in childhood. This is supported by the available data on airway structure in children with wheeze or subsequent asthma (46, 47). Smooth muscle appears in the airways at 5 weeks' gestation (48) and mesenchymal cell tension and paracrine morphogenic factors control the development of the ASM during gestation (49). The various stimuli for increased numbers or size of ASM cells suggest a number of pathways for the development of increased thickness of the layer of ASM as seen in asthma, but little is known about the determinants of hyperplasia or hypertrophy after birth and in patients with asthma.

The small or negligible effects of duration of asthma and age on ASM cell number or size, relative to the effect of asthma group, suggest that the increased cell number or size are predominantly determined early in life or in the history of asthma. Moreover, it seems likely that ASM cell number may contribute to the clinical severity of asthma. Events subsequent to the onset of asthma may result in a small increase in the number of ASM cells, but have little effect on the size of ASM cells. Interventions to prevent these changes in the ASM would need to be investigated early in life.

The functional effects of our findings remain open to speculation. Both moderate and severe cases of asthma are characterized by excessive airway narrowing whereas we have observed hypertrophy in the nonfatal cases (predominantly moderate severity) and hyperplasia in the fatal cases (predominantly severe). This suggests that increased total contractile proteins, either by hypertrophy or by hyperplasia, results in more force development and excessive airway narrowing, as suggested by Oliver and colleagues (50) although certainly in fatal (or clinically severe) cases of asthma hyperplasia contributes most to the increase in muscle mass.

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