Characterization of acinar airspace involvement in asthmatic patients by using inert gas washout and hyperpolarized ^{3}helium magnetic resonance

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Background: The multiple-breath inert gas washout parameter acinar ventilation heterogeneity (S_{acin}) is thought to be a marker of acinar airway involvement but has not been validated by using quantitative imaging techniques in asthmatic patients.
Objective: We aimed to use hyperpolarized ^{3}He diffusion magnetic resonance at multiple diffusion timescales and quantitative computed tomographic (CT) densitometry to determine the nature of acinar airway involvement in asthmatic patients.
Methods: Thirty-seven patients with asthma and 17 age-matched healthy control subjects underwent spirometry, body plethysmography, multiple-breath inert gas washout (with the tracer gas sulfur hexafluoride), and hyperpolarized ^{3}He diffusion magnetic resonance. A subset of asthmatic patients (n = 27) underwent quantitative CT densitometry.
Results: Ninety-four percent (16/17) of patients with an increased S_{acin} had Global Initiative for Asthma treatment step 4 to 5 asthma, and 13 of 17 had refractory disease. The apparent diffusion coefficient (ADC) of ^{3}He at 1 second was significantly higher in patients with S_{acin}-high asthma compared with that in healthy control subjects (0.024 vs 0.017, $P < .05$). S_{acin} correlated strongly with ADCs at 1 second ($R = 0.65$, $P < .001$) but weakly with ADCs at 13 ms ($R = 0.38$, $P < .05$). ADCs at both 13 ms and 1 second correlated strongly with the mean lung density expiratory/inspiratory ratio, a CT marker of expiratory air trapping ($R = 0.77$, $P < .0001$ for ADCs at 13 ms; $R = 0.72$, $P < .001$ for ADCs at 1 second).
Conclusion: S_{acin} is associated with alterations in long-range diffusion within the acinar airways and gas trapping. The precise anatomic nature and mechanistic role in patients with severe asthma requires further evaluation. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Asthma, small airways, acinus, physiology*

Asthma is a chronic inflammatory airway disease characterized by variable airflow obstruction, airway hyperresponsiveness, and structural remodeling in both the large and small airways.[1] Understanding the site and nature of small-airways disease in asthmatic patients is important because it might allow the development of therapies that target this region of the lung or better application of existing therapies, such as extrafine-particle inhalers.[2]

Although it is known that inflammatory and structural changes in asthmatic patients occur in the smaller conducting airways,[3-7] it is not known whether the lesion extends to the more distal intra-acinar airways. The acinar airways of the lung constitute the majority of the airway surface area and comprise the respiratory bronchioles, alveolar ducts, and alveoli.[8] Understanding the role and contribution of the acinar airways to asthma is important because currently available inhaled therapies are not designed to provide penetration to this compartment.[9] A number of tools are available to noninvasively probe the structure of the acinar airways in asthmatic patients. These include the physiologic assessment of gas mixing by using multiple-breath inert gas washout (MBW),[10] measurement of gas diffusion by using hyperpolarized noble gas magnetic resonance techniques,[11] and computed tomographic (CT) densitometry to evaluate expiratory air trapping.[12] However, to date, there has not been a comprehensive assessment of the acinar airways in asthmatic patients using these approaches together.

There are thought to be 2 independent mechanisms of gas-mixing inefficiency in the lungs, namely convection-dependent inhomogeneity (CDI) and diffusion-convection–dependent inhomogeneity (DCDI).[13,14] CDI arises because of unequal convective ventilation between relatively large lung units subtended by conducting airways. DCDI is a more complex mechanism that occurs because of an interaction between convective and diffusive gas flows at the convection-diffusion front, the region of the airway tree at which these flows are of approximately equal

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Abbreviations used
ACQ: Asthma Control Questionnaire
ADC: Apparent diffusion coefficient
CDI: Convection-dependent inhomogeneity
CT: Computed tomography
DCDI: Diffusion-convection–dependent inhomogeneity
FRC: Functional residual capacity
GINA: Global Initiative for Asthma
^{3}He-MR: Hyperpolarized ^{3}helium diffusion magnetic resonance
ICS: Inhaled corticosteroid
Kco: Carbon monoxide transfer coefficient
MBW: Multiple-breath inert gas washout
MLD E/I: Mean lung density expiratory/inspiratory ratio
OCS: Oral corticosteroid
P_{15}: Fifteenth lower percentile of the inspiratory lung attenuation curve
RV: Residual volume
S_{acin}: Acinar ventilation heterogeneity
S_{cond}: Conductive ventilation heterogeneity
SF_6: Sulfur hexafluoride

magnitude. The MBW parameters conductive ventilation heterogeneity (S_{cond}) and acinar ventilation heterogeneity (S_{acin}) were proposed by Verbanck et al[15] as measures of CDI and DCDI, respectively. Because in health the convection-diffusion front is thought to be located within the pulmonary acinus, S_{acin} was proposed as a putative physiologic marker of acinar airspace disease. However, the precise location of the convection-diffusion front is heavily dependent on the molar mass of the inert tracer gas being used, with heavier gases, such as sulfur hexafluoride (SF_6) probing more distal regions of the pulmonary acinus than lighter gases, such as N_2.[10] Increases in S_{acin} have been observed in asthmatic patients, leading to the suggestion that this condition is characterized by a specific structural abnormality in the pulmonary acinus.[16] However, the precise nature of this structural abnormality has not been elucidated.

Hyperpolarized ^{3}helium diffusion magnetic resonance (^{3}He-MR) is a technique that allows microstructural changes at the level of the alveoli and acinar airways to be examined noninvasively under resting physiologic conditions.[11] The apparent diffusion coefficient (ADC) of ^{3}He within the pulmonary acinus can be measured across a wide range of timescales, from 1 ms to 10 seconds. Short or intermediate timescales of the order of a few milliseconds correspond to diffusion within a single alveolus or a single acinar airway, respectively, whereas long timescales of the order of seconds correspond to diffusion within several acinar airways,[11] as illustrated in Fig 1.[17] ^{3}He-MR has been extensively validated against histology in both human subjects and animal models of disease. Several studies have shown that short-timescale ^{3}He or ^{129}Xe ADCs are increased in both patients with emphysema[18-24] and animal models of emphysema[25-28] in comparison with values obtained in healthy lungs. Moreover, in a number of these studies, ADCs were found to correlate with quantitative histologic measures of emphysema, such as the mean linear intercept, mean alveolar internal area, and mean chord length.[21,23,25-28] Air trapping can be assessed by using physiologic measurements of lung volumes[29] or with imaging techniques, such as quantitative CT densitometry.[12] Indeed, we have recently identified CT imaging phenotypes of asthma using these approaches and identified that air trapping is a feature of all CT imaging clusters and is associated with more severe disease.[30]

We aimed to use ^{3}He-MR at multiple diffusion timescales and quantitative CT densitometry to determine the structural correlates of the multiple-breath washout marker S_{acin} in asthmatic patients using SF_6-MBW. We hypothesized that (1) asthmatic patients with an increased S_{acin} would manifest altered long-range diffusion suggestive of intra-acinar airway disease and (2) the degree of acinar involvement in asthma would be independent of lung hyperinflation.

METHODS

Thirty-seven patients with asthma and 17 age-matched healthy control subjects were recruited. All of the patients within this study were recruited from our secondary care asthma center (Glenfield Hospital, Leicester, United Kingdom). The center primarily evaluates patients at Global Initiative for Asthma (GINA) treatment steps 3 to 5 to optimize their disease control and any potential comorbidities (eg, rhinosinusitis) and treatment nonadherence. Some of these patients (steps 4-5) were evaluated in a difficult/complex asthma clinic that evaluates treatment-refractory populations. Therefore our recruited population was representative of a secondary care asthmatic population in the United Kingdom and included patients with treatment-refractory disease.

Patients were seen in the stable state, with no changes having been made to their regular inhaled or oral asthma therapy within the preceding 6 weeks. All participants were never smokers or exsmokers with a smoking history of less than 10 pack years. Asthma was diagnosed in a secondary care setting according to British Thoracic Society guidelines.[31] The study was approved by the National Research and Ethics Committee (East Midlands, Leicester, United Kingdom), and all participants provided written informed consent.

Patients with asthma completed the 6-point Asthma Control Questionnaire (ACQ-6)[32] and the Standardized Asthma Quality of Life Questionnaire.[33] Participants were administered 200 μg of salbutamol through a metered-dose inhaler and spacer to minimize the confounding effects of airway smooth muscle tone on physiologic and imaging assessments. Spirometry, body plethysmography, and measurement of carbon monoxide diffusing capacity were performed according to American Thoracic Society/European Respiratory Society guidelines.[34-36] Predicted values and standardized residuals (z scores) were derived by using the Global Lung Function Initiative (2012) equations for spirometry[37] and the European Community for Steel and Coal (1993) equations for lung volumes and carbon monoxide transfer coefficient (Kco).[38] Induced sputum inflammatory cell counts were obtained in asthmatic patients by using a previously published method.[39]

MBW was performed according to current guidelines[40] by using the SF_6 wash-in method described by Horsley et al.[41] SF_6 was chosen as the inert tracer gas because of its heavy molar mass and based on previous simulation data from Dutrieue et al[17] suggesting that phase III slope sensitivity to SF_6 is maximal at the level of the alveolar duct (generations 20-21, Fig 1). Participants wore a nose clip and breathed an air mixture containing 0.2% SF_6, while respiratory flows and exhaled breath SF_6 concentrations were monitored with an Innocor photoacoustic gas analyzer (Innovision A/S, Odense, Denmark). Participants maintained a steady respiratory rate of approximately 12 breaths per minute and a constant tidal volume of 1 L throughout the test by using a real-time visual display of inspired volume as a guide. Once inhaled and exhaled SF_6 concentrations had equalized, participants were switched to breathing room air during an expiration. The test was terminated when the end-tidal concentration of SF_6 in exhaled breath decreased to less than 1/40th of the original concentration for 3 consecutive breaths. Lung clearance index,[10] S_{cond}, and S_{acin}[15] were calculated by using custom software written with TestPoint (Measurement Computing Corp, Norton, Mass).

^{3}He-MR was performed with a 0.15-T permanent magnet system (Intermagnetics General Corp, New York, NY) and a Surrey Medical Imaging Systems console (Surrey, United Kingdom). Participants were scanned in the supine position and inhaled 600 mL of a ^{3}He/^{4}He mixture from functional residual capacity (FRC), followed by a breath-hold lasting between 2 and 10

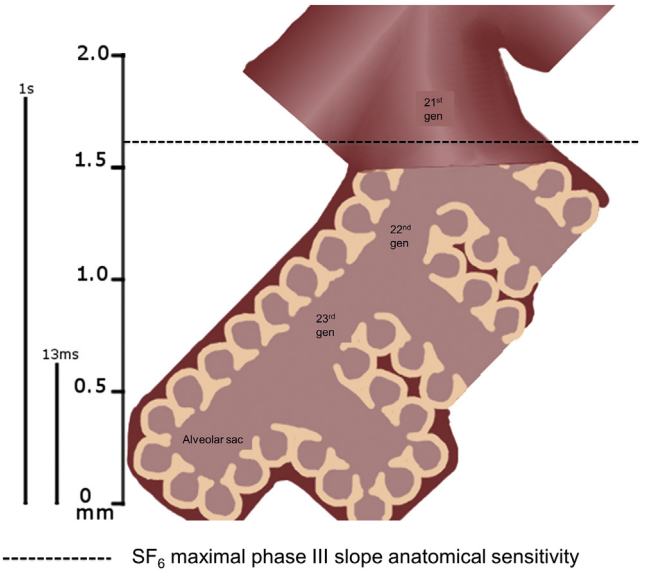

FIG 1. Schematic diagram of length scales probed by helium ADCs. The *dotted line* represents maximal anatomic sensitivity of SF_6 phase III slopes.[17]

seconds depending on the pulse sequence being performed. Intermediate-timescale ADCs (13 ms) were measured by using a diffusion-weighted Carr-Purcell-Meiboom-Gill technique,[42,43] and long-timescale ADCs (1 second) were measured by using a stimulated echo sequence.[44] The first 7 asthmatic patients and the first 2 healthy control subjects to enter the study took part in a pilot phase in which only intermediate-timescale ADC measurements were made.

The effect of lung volume changes on intermediate-timescale ADCs have been previously reported, with a strong positive correlation observed between the degree of lung inflation and the 13-ms ADC.[43] To aid the interpretation of our results, we also investigated the relationship between lung volume and long-timescale ADCs in 3 healthy control subjects and 3 asthmatic patients. Long-timescale ADC measurements were performed at specified lung volumes above either residual volume (RV) or FRC. The absolute values of RV and FRC were determined by using body plethysmography.

A subset of asthmatic patients (n = 27) was further characterized by using quantitative CT densitometry. Volumetric whole-lung scans were obtained with a Siemens Sensation 16 scanner with the following low-dose protocol: 16 × 0.75–mm collimation, 1.5-mm pitch, 120 kVp, 40 mA, 0.5-second rotation time, and scanning field of view of 500 mm, with dose modulation off. Scans were obtained at full inspiration and full expiration. Images were reconstructed with a slice thickness of 0.75 mm at a 0.5-mm interval by using B35f kernal. VIDA Apollo image analysis software (VIDA Diagnostics, Coralville, Iowa) was used for quantitative analysis of lung densitometry. The main parameters extracted were the mean lung density expiratory/inspiratory ratio (MLD E/I), a marker of expiratory air trapping,[45] and the fifteenth lower percentile of the inspiratory lung attenuation curve (P_{15}), a marker of emphysema.[46]

Statistical analyses were performed with SPSS 20 (IBM, Somers, NY) and Prism 6 (GraphPad Software, La Jolla, Calif) software. Group comparisons were performed with the Student *t* test, 1-way ANOVA with the Tukey test for multiple comparisons, or the Mann-Whitney *U* test for continuous variables and the Fisher exact test or χ^2 test for proportions. Relationships between continuous variables were investigated by using the Pearson correlation coefficient. Previous data on the group SD of ADCs at 1 second were not available for use in a sample size calculation. However, Wang et al[21] reported a 0.0051 cm^2s^{-1} difference in mean ADCs at 1.5 seconds between the healthy and asthmatic groups, with a group SD of 0.0026 cm^2s^{-1} in the healthy group and 0.0055 cm^2s^{-1} in the asthmatic group using similar methodology to our own. We calculated that to detect this difference between the healthy and asthmatic groups at 90% power using a *t* test with a 5% significance level, we would require 15 patients in each group.

RESULTS

Asthmatic patient–reported and clinical outcomes in patients with increased S_{acin}

Table I[47,48] shows the demographic and clinical characteristics of the participant groups. Patients with asthma were divided into S_{acin}-normal and S_{acin}-high groups, with the upper limit of normal for S_{acin} being defined as the mean + 1.64 SDs in the age-matched control group (0.204 L^{-1}). The 3 groups were well matched for age and sex. The S_{acin}-high group had evidence of suboptimal asthma control, with significantly higher ACQ-6 scores compared with the S_{acin}-normal group. In addition, 76% of the S_{acin}-high group had evidence of refractory asthma ($P < .05$ vs those with S_{acin}-normal asthma), according to the American Thoracic Society criteria,[48] with the majority (n = 16/17) having GINA treatment step 4 to 5 asthma.[47] In contrast, 45% of the S_{acin}-normal group had refractory disease, with patients belonging to the full spectrum of GINA treatment steps.

Physiologic phenotyping of asthmatic patients with an increased S_{acin}

Table II shows physiologic parameters in the participant groups. The S_{acin}-high group exhibited significantly worse expiratory flow limitation and expiratory air trapping than the S_{acin}-normal group. FEV_1 (percent predicted) was significantly lower in the S_{acin}-high group compared with the S_{acin}-normal group (69.3% vs 90.9%, $P < .01$), and the RV/total lung capacity ratio was significantly higher (48.3% vs 38.2%, $P < .01$), as was the FRC (percent predicted; 131.5% vs 103.7%, $P < .01$). K_{CO} did not differ significantly between the groups.

Imaging-based phenotyping of asthmatic patients with an increased S_{acin}

Fig 2 shows the CT densitometric data in the 2 asthma groups. There was evidence of expiratory air trapping in the S_{acin}-high group, with a significantly increased MLD E/I compared with the S_{acin}-normal group (0.89 vs 0.83, $P < .05$). However, the inspiratory P_{15} did not differ between the groups, suggesting that an increased S_{acin} is not associated with CT density–based assessments of emphysema in asthmatic patients.

Fig 3 shows the intermediate- and long-timescale ADC measurements across the 3 groups. ADCs at 1 second were significantly higher in the S_{acin}-high group compared with those in the healthy control group (0.024 vs 0.017, $P < .05$), with a trend toward a significant difference between the S_{acin}-high and S_{acin}-normal asthmatic groups (0.024 vs 0.019, $P = .09$). There was no evidence that acinar airway disease was attenuated by systemic corticosteroid therapy. In particular, mean S_{acin} was 0.256 L^{-1} in patients taking long-term oral corticosteroids (OCSs) compared with 0.191 L^{-1} in those not taking OCSs ($P > .05$). Mean ADCs at 13 ms were 0.121 cm^2s^{-1} in patients taking OCSs and 0.131 cm^2s^{-1} in patients not taking OCSs ($P > .05$), whereas mean ADCs at 1 second were 0.023 cm^2s^{-1} and 0.021 cm^2s^{-1}, respectively ($P > .05$).

TABLE I. Demographic and clinical characteristics of participant groups

	Healthy control subjects (n = 17)	Patients with S_{acin}-normal asthma (n = 20)	Patients with S_{acin}-high asthma (n = 17)
Age (y)	53.4 (3.3)	54.2 (3.1)	61.2 (1.9)
Sex (% male)	47	40	65
Height (cm)	170.6 (2.6)	164.8 (2.5)	169.7 (1.9)
Weight (kg)*	75.0 (2.7)	78.1 (3.3)	90.4 (5.0)‡
Body mass index (kg/m^2)*	25.8 (0.8)	28.9 (1.3)	31.2 (1.4)§
Smoking status			
Never smokers, no. (%)	15 (88)	15 (75)	10 (59)
Exsmokers, no. (%)	2 (12)	5 (25)	7 (41)
Pack years, median (range)	0 (0-2)	0 (0-7)	0 (0-8)
Age of onset of asthma symptoms (y)	—	23.4 (5.0)	27.5 (5.3)
Duration of asthma (y)	—	30.9 (3.8)	33.7 (5.1)
Atopic status (% positive)	—	85	82
ACQ-6 score*	—	1.43 (0.26)	2.14 (0.22)
AQLQ(S) score†	—	5.61 (0.23)	4.95 (0.31)
Sputum neutrophil count (%)	—	57.2 (6.0)	61.8 (7.1)
Sputum eosinophil count (%)‖	—	2.69 (1.23-5.89)	1.76 (0.76-4.04)
Blood eosinophil count ($\times 10^9$/L)	—	0.33 (0.04)	0.34 (0.07)
Daily dose of ICS (beclomethasone dipropionate equivalent [μg])	—		
Median		1000	1600
Range		0-2000	200-2000
Use of long-acting β-agonists (% of subjects)	—	75	94
Regular use of oral prednisolone (% of subjects)	—	20	35
Use of leukotriene receptor antagonist (% of subjects)	—	10	35
Use of methylxanthine (% of subjects)†	—	10	41
Asthma treatment step¶†	—	1:6:9:4	1:0:9:7
Refractory asthma (% positive)#†	—	45	76

Data are expressed as mean (SEs) or proportions, unless stated otherwise.
AQLQ(S), Standardized Asthma Quality of Life Questionnaire.
Groups were compared by using 1-way ANOVA with the Tukey test for multiple comparisons or the Student *t* test for parametric data, Mann-Whitney *U* test for nonparametric data, and χ^2 or Fisher exact tests for proportions. Significant differences across or between groups are denoted as follows: $*P < .05$. Trends toward significance are denoted as follows: $\dagger P < .1$. Significant differences compared with the healthy control group are denoted as follows $\ddagger P < .05$ or $\S P < .01$.
‖Expressed as geometric mean (95% CI). Log-transformed data were compared between groups by using the Student *t* test.
¶As defined by GINA[47] and expressed as the number of patients receiving treatment at step 2:step 3:step 4:step 5.
#Refractory asthma was defined according to the American Thoracic Society Workshop definition.[48]

Evaluation of the contribution of lung volume to ADCs

Fig 4 shows correlations between ADCs and S_{acin} (Fig 4, *A* and *B*), FRC (percent predicted; Fig 4, *C* and *D*), and MLD E/I (Fig 4, *E* and *F*) in asthmatic patients. S_{acin} correlated weakly with ADCs at 13 ms ($R = 0.38$, $P < .05$) but strongly with ADCs at 1 second ($R = 0.65$, $P < .001$). S_{cond} did not correlate significantly with ADCs at either 13 ms ($R = -0.037$, $P > .05$) or 1 second ($R = 0.101$, $P > .05$), indicating that ADCs are related specifically to the acinar component of ventilation heterogeneity.

ADCs at both 13 ms and 1 second correlated strongly with FRC percent predicted ($R = 0.73$, $P < .0001$ for ADCs at 13 ms; $R = 0.68$, $P < .0001$ for ADCs at 1 second) and with the MLD E/I, a CT marker of expiratory air trapping ($R = 0.77$, $P < .0001$ for ADCs at 13 ms; $R = 0.72$, $P < .0001$ for ADCs at 1 second). However, in healthy subjects there were no significant correlations between ADCs at 13 ms/1 second and either S_{acin} or FRC (percent predicted).

Fig 5 shows the relationship between lung inflation and ADCs at 1 second in 3 healthy volunteers (Fig 5, *A*) and 3 patients with asthma (Fig 5, *B*). The correlation was positive but weak in both cases, only reaching statistical significance in the asthmatic patients ($P < .05$). The slope of the lines was shallow, with a 50% increase in lung inflation resulting in a 3.7% increase in ADCs in healthy volunteers and a 4.5% increase in asthmatic patients.

DISCUSSION

The main finding of this study is that in asthmatic patients the MBW parameter S_{acin}, determined by using the tracer gas SF_6, is strongly associated with increases in long-timescale ADCs. However, this association is not observed in healthy subjects. Moreover, increases in long-timescale ADCs cannot be reproduced purely by lung inflation, suggesting that such increases result from a specific structural abnormality in the pulmonary acinus in asthmatic patients.

A number of previous studies have investigated the clinical significance of the acinar lesion in asthmatic patients. Farah et al[49] found that improvements in S_{acin} were independently associated with improvements in 5-point ACQ scores after the initiation of inhaled corticosteroid (ICS) treatment and that markers of ventilation heterogeneity could predict the response to ICS dose titration.[50] Thompson et al[51] found that S_{acin} correlated with asthma severity, as measured by using GINA treatment steps and that asthma exacerbations were associated with increases in S_{acin}. We observed in the present study that an increased S_{acin} was present primarily in patients with severe (GINA 4-5) asthma and in approximately 75% of cases of refractory asthma. These observations might reflect a higher prevalence of severe and refractory asthma in our study population; however, the proportion of patients with refractory asthma was significantly higher among patients with S_{acin}-high asthma when compared with those with

TABLE II. Physiologic, CT, and magnetic resonance data across participant groups

	Healthy control subjects (n = 17)	Patients with S_{acin}-normal asthma (n = 20)	Patients with S_{acin}-high asthma (n = 17)
FEV_1 (% predicted)§	97.7 (3.4)	83.9 (3.8)‖	65.4 (4.8)**‡‡
FEV_1 *z* score§	−0.18 (0.23)	−1.16 (0.26)‖	2.28 (0.29)**††
FVC (% predicted)†	107.7 (3.7)	94.4 (2.9)‖	91.6 (3.3)¶
FVC *z* score†	0.53 (0.27)	−0.42 (0.21)‖	−0.66 (0.26)¶
FEV_1/FVC (%)§	72.1 (1.7)	70.6 (2.5)	55.6 (3.3)#§§
FEV_1/FVC *z* score‡	−1.03 (0.23)	−1.23 (0.30)	−2.73 (0.31)#‡‡
FRC (L)†	3.67 (0.26)	3.08 (0.23)	4.28 (0.26)‡‡
FRC (% predicted)*	114.4 (6.2)	103.7 (6.7)	131.5 (6.2)‡‡
TLC (L)	6.92 (0.48)	5.70 (0.34)	6.77 (0.39)
TLC (% predicted)	109.8 (3.7)	103.3 (3.8)	109.5 (3.4)
RV/TLC ratio (%)§	31.9 (2.2)	38.2 (1.9)	48.3 (2.3)**‡‡
RV/TLC ratio (% predicted)§	88.3 (3.6)	104.8 (4.2)	125.6 (6.0)**‡‡
V_A/TLC (%)§	88.2 (1.8)	82.0 (1.9)	74.3 (1.9)**††
K_{CO} ($mmol \cdot min^{-1} \cdot kPa^{-1} \cdot L^{-1}$)	1.55 (0.06)	1.66 (0.06)	1.58 (0.07)
LCI§	7.34 (0.26)	7.43 (0.25)	9.59 (0.31)**‖‖
S_{cond} (L^{-1})	0.029 (0.004)	0.054 (0.015)	0.068 (0.012)
S_{acin} (L^{-1})§	0.120 (0.012)	0.115 (0.011)	0.319 (0.026)**‖‖
MLD E/I	—	0.83 (0.02)	0.89 (0.01)
P_{15} (HU)	—	−941 (5)	−950 (5)
ADC, 13 ms ($cm^2 s^{-1}$)	0.132 (0.006)	0.121 (0.005)	0.137 (0.005)
ADC, 1 s ($cm^2 s^{-1}$)	0.017 (0.001)	0.019 (0.001)	0.024 (0.002)

Data were expressed as means (SEs).

FVC, Forced vital capacity; *LCI*, lung clearance index; *TLC*, total lung capacity; V_A, alveolar volume from single breath helium dilution.

Groups were compared by using 1-way ANOVA with the Tukey test for multiple comparisons or the Student *t* test. Significant differences across groups are denoted as follows: *$P < .05$), †$P < .01$, ‡$P < .001$, or §$P < .0001$. Significant differences compared with the healthy control group are denoted as follows: ‖$P < .05$, ¶$P < .01$, #$P < .001$, or **$P < .0001$. Significant differences between the S_{acin}-low and S_{acin}-high asthmatic groups are denoted as follows: ††$P < .05$, ‡‡$P < .01$, §§$P < .001$, or ‖‖$P < .0001$.

S_{acin}-normal asthma. In contrast, we did not find a similar association with S_{cond}. These observations might suggest that the acinar abnormality captured with SF_6 gas washout in asthmatic patients might be refractory to conventional pharmacotherapy. In support of this hypothesis is a recent interventional study in which switching standard ICSs to small-particle ICSs had no significant effect on S_{acin} in asthmatic patients.[52] However, large and appropriately powered intervention studies would be required to confirm this hypothesis fully.

The acinar airways form an asymmetrically dichotomous branching network in 3-dimensional space that can be described in terms of its mean airway radius, branch length, and branch angle. In this study we performed MBW by using the tracer gas SF_6 because of its large molar mass,[40] thus increasing the likelihood of probing the pulmonary acinus. Modeling studies by Dutrieue et al[17] have indicated that the convection-diffusion front with SF_6 is likely to occur at the level of the alveolar duct (generations 20-21). The diffusion of helium in an acinar airway is more restricted in the transverse direction than in the longitudinal direction, and therefore at short or intermediate timescales, such as 13 ms, the ^{3}He ADC is more sensitive to airway radius than airway branch length. Long-timescale ADCs are a measure of the network properties of the acinar airways, with higher values being associated with a greater number of interacinar and intra-acinar connections. Simulations of long-timescale ADCs within an anatomically realistic asymmetrically dichotomous model of the acinus yielded values that were of the same order as those observed experimentally in healthy subjects,[53] whereas the addition of intra-acinar collateral channels to the model produced significantly increased simulated long-timescale ADCs.[54] An increase in airway branch length causes an increase in long-timescale ADCs because it allows greater longitudinal displacement of helium atoms along the airway axes. Long-timescale ADCs might also be affected by the width of the alveolar sleeve surrounding the acinar airways, with an increase in sleeve width causing a reduction in axial diffusion and a consequent reduction in long-timescale ADCs.[55]

An important question to address is whether the correlation between S_{acin} and long-timescale ADCs represents a true structural change in the pulmonary acinus or whether the relationship is driven by the presence of expiratory air trapping and hyperinflation in patients with raised S_{acin}. Hajari et al[56] used ^{3}He-MR lung morphometry to assess the changes that occur in the acinar airways during lung inflation in healthy subjects. They concluded that lung inflation occurs primarily through alveolar recruitment and, to a lesser extent, through expansion of alveolar ducts. Alveolar sleeve width actually decreased with increasing lung inflation. The expansion of alveolar ducts would be expected to increase short- or intermediate-timescale ADCs, and indeed, it is known that the 13-ms ADC has a strong linear relationship with lung inflation in healthy subjects.[43] However, we observed only minor effects of lung inflation on long-timescale ADCs, suggesting that hyperinflation alone cannot account for the strong association between S_{acin} and long-timescale ADCs.

We observed strong correlations between the CT marker of expiratory air trapping (MLD E/I) and both intermediate and long-timescale ADCs, suggesting that there might be common structural abnormalities at the level of the acinar airways that result in both expiratory air trapping and altered diffusion in the distal airspaces. A possible method of elucidating these abnormalities in future studies might be micro-CT of surgical lung biopsy specimens or resected lung specimens, as has been performed in patients with COPD.[57] We found no evidence of emphysema in patients with asthma and increased S_{acin}, with

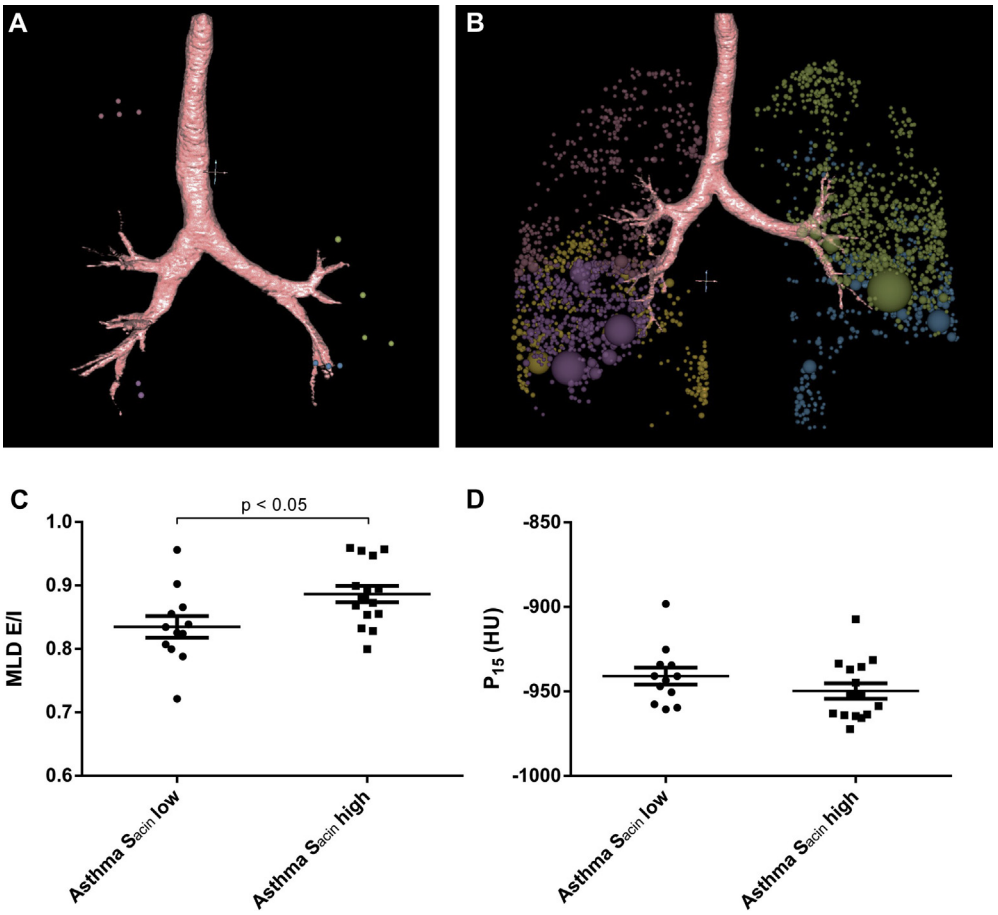

FIG 2. Quantitative CT densitometry between asthma groups. **A** and **B,** Quantitative assessment of expiratory air trapping in patients with low and high levels of air trapping, respectively. *Colored spheres* represent lung areas greater than 1 mL in volume with an attenuation on expiratory scans of less than −856 Hounsfield units *(HU)*. **C** and **D,** MLD E/I and P_{15}, respectively, in asthmatic patients. *Error bars* indicate means ± SEMs.

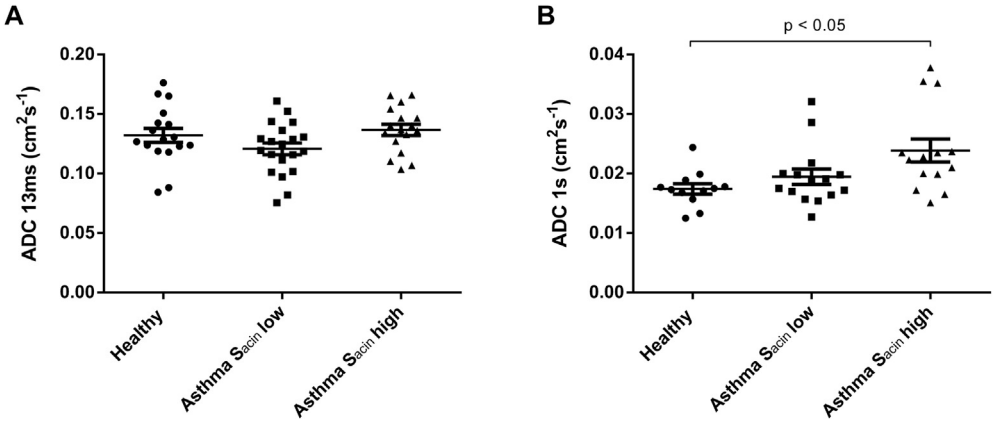

FIG 3. ADCs across groups. ADCs at 13 ms **(A)** and 1 second **(B)** are shown across healthy and asthmatic groups. *Error bars* indicate means ± SEMs.

neither P_{15} nor KCO differing between the S_{acin}-normal and S_{acin}-high groups. Verbanck et al[16] also observed normal KCO values in asthmatic patients, an observation later confirmed by our own group,[58] suggesting that the alveolar-capillary membrane remains intact in this condition. However, there is some evidence that lung elastic recoil is reduced in asthmatic patients,[59,60] and autopsy studies have suggested that this might be due to a subtle breakdown of lung architecture.[61,62]

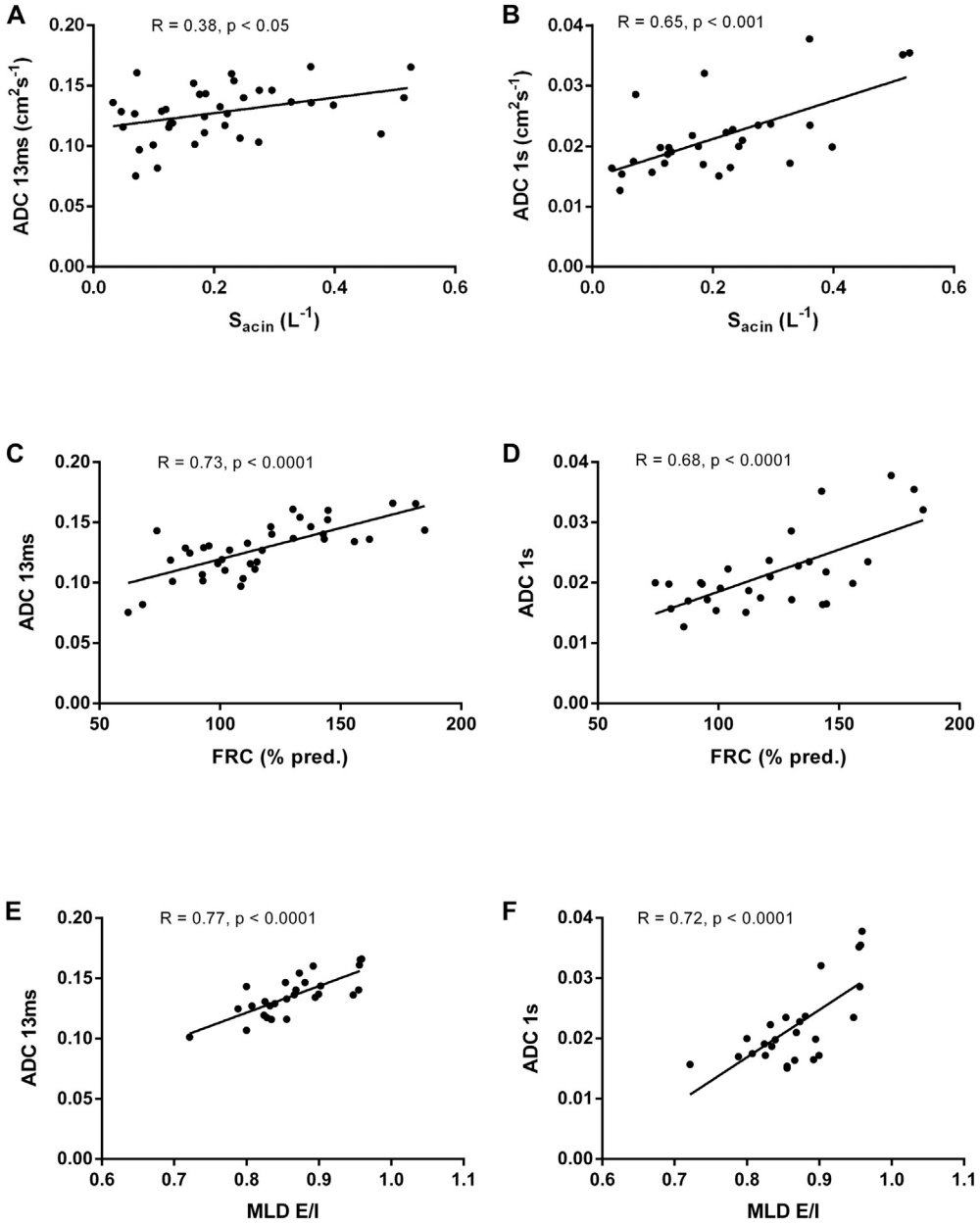

FIG 4. Correlations between ^{3}He-MR, CT, and physiologic variables in asthmatic patients. Correlations are shown between ADCs and S_{acin} (**A** and **B**), FRC percent predicted (**C** and **D**), and MLD E/I (**E** and **F**). Best-fit linear regression lines and Pearson correlation coefficients are shown.

Our study has a number of potential limitations. First, patients were drawn predominantly from a secondary care center, and as such, the results might not be generalizable to an unselected asthmatic population. In particular, our study group was likely to have a higher proportion of patients with refractory asthma and those taking long-term OCSs than a general asthmatic population. However, it is important to note that the rationale for referral to our asthma center and difficult asthma clinic included optimization of other factors, such as treatment nonadherence, rhinosinusitis, and psychological/behavioral issues. As such, the selected population was not specifically identified from a treatment-refractory cohort.

Second, the mean age of patients in our study was 57.4 years, which might be slightly older than an unselected asthmatic population, and the proportion of male subjects among our asthmatic group was 51.4% compared with 36.9% in a previously published large cohort with difficult asthma.[63]

Third, it has been shown that the pulmonary acinus might be sensitive to cigarette smoking.[64] Therefore, despite the fact that real-world asthmatic populations include both exsmokers and current smokers, we chose to recruit only never smokers or exsmokers with a smoking history (<10 pack years) to this study. This was done deliberately because we wanted to explore the relationship between a noninvasive marker of small-airway dysfunction and airway diffusion measurements in asthmatic patients, and our measurements would have been confounded by smoking-associated acinar changes if we had selected smoking and heavy exsmoking populations.

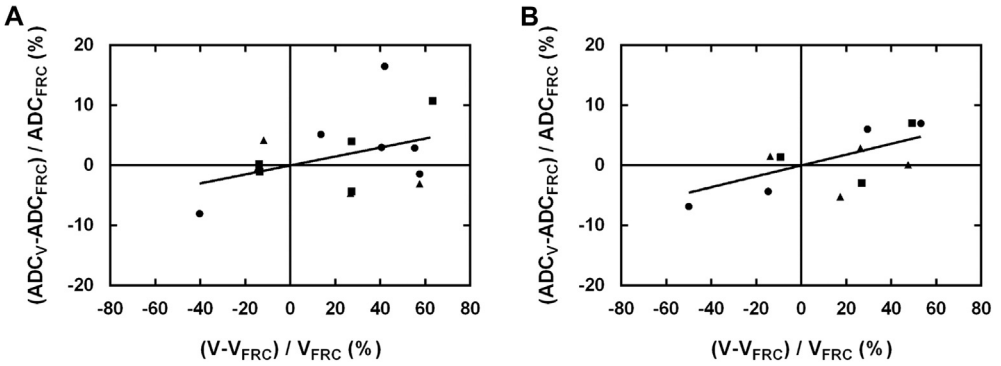

FIG 5. Change in ADCs (percentage) against change in volume of gas in the lungs (percentage) in healthy subjects and asthmatic patients. Correlations are shown between percentage change in ADC and percentage change in volume of gas in the lungs in 3 healthy subjects **(A)** and 3 asthmatic patients **(B)**. The 3 participants in each case are denoted with different symbols. ADC_{FRC}, ADC at FRC (extrapolated); ADC_V, ADC at lung volume V; V, volume of gas in lungs; V_{FRC}, volume of gas in lungs at FRC.

However, it remains possible that differences in low-grade cigarette smoke exposure might have accounted for some of the proposed structural changes observed within the acinus in the S_{acin}-high population.

Finally, a further potential limitation of the study was that the MR pulse sequences used did not provide 3-dimensional spatial information. Future studies incorporating 3-dimensional spatial encoding of ADCs might provide further insights into the structural correlates of inert gas washout indices.

We conclude that the MBW parameter S_{acin} appears to be associated with a structural abnormality in the pulmonary acinus in asthmatic patients, causing subtle alterations in diffusion within the acinar airways. In addition, the proportion of patients with refractory asthma and a high S_{acin} was significantly greater when compared with that of patients with a normal S_{acin}, suggesting that the lesion might be clinically important. However, this latter observation must be regarded as exploratory and warrants further prospective evaluation.

Longitudinal studies are required to determine the long-term prognostic significance of acinar airway disease in asthmatic patients and whether it might be amenable to fine-particle inhaled or systemic therapies.

Clinical implications: There is evidence of a structural abnormality in the pulmonary acinus in asthmatic patients, which is present primarily in those with severe disease.

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