Characterisation of Acinar Airspace Involvement in Asthma using Inert Gas Washout and Hyperpolarised $^3$Helium Magnetic Resonance

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Abstract

Background
The multiple breath washout (MBW) parameter $S_{acin}$ is thought to be a marker of acinar airway involvement, but has not been validated using quantitative imaging techniques in asthma.

Objective
We aimed to utilise $^3$He diffusion magnetic resonance ($^3$He-MR) at multiple diffusion timescales and quantitative computed tomography (CT) densitometry to determine the nature of acinar airway involvement in asthma.

Methods
Thirty-seven patients with asthma and seventeen age-matched healthy controls underwent spirometry, body plethysmography, MBW (using the tracer gas sulphur hexafluoride) and $^3$He-MR. A subset of patients with asthma (n = 27) underwent quantitative CT densitometry.

Results
Ninety-four percent (16/17) of patients with an elevated $S_{acin}$ had GINA treatment steps 4/5 asthma and 13/17 had refractory disease, and $S_{acin}$ was a significant predictor of refractory asthma. The apparent diffusion coefficient (ADC) of $^3$He at 1s was significantly higher in patients with $S_{acin}$-high asthma compared to healthy controls (0.024 vs 0.017, $p < 0.05$). $S_{acin}$ correlated strongly with ADC at 1s ($R = 0.65$, $p < 0.001$), but weakly with ADC at 13ms ($R = 0.38$, $p < 0.05$). ADC at both 13ms and 1s correlated strongly with the mean lung density.
expiratory / inspiratory ratio, a CT marker of expiratory air trapping (R = 0.77, p < 0.0001 for ADC at 13ms; R = 0.72, p < 0.001 for ADC at 1s).

Conclusion

$S_{acin}$ is associated with refractory asthma phenotype and alterations in long-range diffusion within the acinar airways and gas trapping. The precise anatomical nature and mechanistic role in refractory disease severe asthma requires further evaluation.

Key words: Asthma, small airways, acinus, physiology
Clinical implications:

There is evidence of a structural abnormality in the pulmonary acinus in patients with asthma, which is associated with refractory disease present primarily in severe disease.

Capsule summary:

Quantitative imaging techniques are utilised to determine the structural correlates of $S_{acini}$, a putative physiological marker of acinar airway disease. The results suggest a structural abnormality in this region is associated with refractory disease which warrants further clinico-pathological evaluation.
Abbreviations:

\(^3\)He-MR   Hyperpolarised \(^3\)helium diffusion magnetic resonance
ACQ    Asthma Control Questionnaire
ADC    Apparent diffusion coefficient
AQLQ(S) Standardised Asthma Quality of Life Questionnaire
BMI    Body mass index
CDI    Convection-dependent inhomogeneity
CT     Computed tomography
DCDI   Diffusion-convection-dependent inhomogeneity
FRC    Functional residual capacity
ICS    Inhaled corticosteroids
KCO    Carbon monoxide transfer coefficient
MBW    Multiple breath inert gas washout
MLD E/I Mean lung density expiratory / inspiratory ratio
\(P_{15}\) Fifteenth lower percentile of the inspiratory lung attenuation curve
ROC    Receiver operating characteristic
RV     Residual volume
\(S_{\text{acin}}\) Acinar ventilation heterogeneity
\(S_{\text{cond}}\) Conductive ventilation heterogeneity
TLC    Total lung capacity
Introduction

Asthma is a chronic inflammatory airway disease that is characterised by variable airflow obstruction, airway hyperresponsiveness and structural remodelling in both the large and small airways [1]. Understanding the site and nature of small airways disease in asthma is important as it may allow the development of therapies that target this region of the lung or better application of existing therapies such as extra fine particle inhalers [2].

While it is known that inflammatory and structural changes in asthma 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 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 non-invasively probe the structure of the acinar airways in patients with asthma. These include the physiological assessment of gas mixing using multiple breath inert gas washout (MBW) [10], measurement of gas diffusion using hyperpolarised noble gas magnetic resonance techniques [11], and computed tomography (CT) densitometry to evaluate expiratory air trapping [12]. However to date there has not been a comprehensive assessment of the acinar airways in asthma using these approaches together.

There are thought to be two independent mechanisms of gas mixing inefficiency in the lungs, namely convection-dependent inhomogeneity (CDI) and diffusion-convection-dependent inhomogeneity (DCDI) [13,14]. CDI arises due to unequal convective ventilation between relatively large lung units subtended by conducting airways. DCDI is a more complex
mechanism that occurs due to 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 magnitude. The MBW parameters $S_{\text{cond}}$ and $S_{\text{acin}}$ were proposed by Verbanck et al. as measures of CDI and DCDI, respectively [15]. Since in health, the convection-diffusion front is thought to be located within the pulmonary acinus, $S_{\text{acin}}$ was proposed as a putative physiological marker of acinar airspace disease. However, the precise location of the convection-diffusion front is heavily dependent upon the molar mass of the inert tracer gas being used, with heavier gases such as sulphur hexafluoride (SF$_6$) probing more distal regions of the pulmonary acinus than lighter gases such as N$_2$ [10]. Elevations in $S_{\text{acin}}$ have been observed in patients with asthma, leading to the suggestion that this condition is characterised by a specific structural abnormality in the pulmonary acinus [16]. However, the precise nature of this structural abnormality has not been elucidated.

Hyperpolarised $^3$He diffusion magnetic resonance ($^3$He-MR) is a technique that allows microstructural changes at the level of alveoli and acinar airways to be examined non-invasively, under resting physiological conditions [11]. The apparent diffusion coefficient (ADC) of $^3$He within the pulmonary acinus may be measured across a wide range of timescales, from 1ms to 10s. Short or intermediate timescales of the order of a few milliseconds correspond to diffusion within a single alveolus or a single acinar airway, respectively, while long timescales of the order of seconds correspond to diffusion within several acinar airways [11], as illustrated in Figure 1. $^3$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-ADC is elevated in both patients with emphysema [17-23], and in animal models of emphysema [24-27], in comparison with values obtained in healthy lungs. Moreover, in a number of these studies ADC was found to correlate with
quantitative histological measures of emphysema such as the mean linear intercept, mean alveolar internal area and mean chord length [20, 22, 24-27]. Air trapping may be assessed using physiological measurements of lung volumes [28], 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 [29].

We aimed to utilise $^3$He-MR at multiple diffusion timescales and quantitative CT densitometry to determine the structural correlates of the multiple breath washout marker $S_{acin}$ in asthma, using SF$_6$-MBW. We hypothesised that (i) asthma patients with an elevated $S_{acin}$ would manifest altered long range diffusion suggestive of intra-acinar airway disease, and (ii) the degree of acinar involvement in asthma would be independent of lung hyperinflation. In addition, we sought to explore the clinical significance of our findings by determining whether markers of acinar airway disease were predictive of a treatment refractory asthma phenotype in our patient cohort.

**Methods**

Thirty-seven patients with asthma and seventeen age-matched healthy control subjects were recruited. All of patients within this study were recruited from our secondary care asthma centre [Glenfield Hospital; Leicester]. The centre primarily evaluates patients at GINA treatment steps 3-5 to optimise their disease control and any potential comorbidities e.g. rhino sinusitis, treatment non adherence. 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 asthma population in the UK 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 six weeks. All participants were never smokers or ex-smokers with less than 10 pack years’ smoking history. Asthma was diagnosed in a secondary care setting according to British Thoracic Society guidelines [30]. The study was approved by the National Research and Ethics Committee – East Midlands, Leicester, and all participants gave their written informed consent.

Patients with asthma completed the six-point Asthma Control Questionnaire (ACQ-6) [31] and the standardised Asthma Quality of Life Questionnaire (AQLQ(S)) [32]. Participants were administered 200 micrograms of salbutamol via a metered-dose inhaler and spacer, to minimise the confounding effects of airway smooth muscle tone on physiological and imaging assessments. Spirometry, body plethysmography and measurement of carbon monoxide diffusing capacity were performed according to American Thoracic Society (ATS) / European Respiratory Society guidelines [33-35]. Predicted values and standardised residuals (z scores) were derived using the Global Lung Function Initiative (2012) equations for spirometry [36], and the European Community for Steel and Coal (1993) equations for lung volumes and carbon monoxide transfer coefficient [37]. Induced sputum inflammatory cell counts were obtained in patients with asthma using a previously published method [38].

MBW was performed according to current guidelines [39] using the SF6 wash-in method described by Horsley et al [40]. SF6 was chosen as the inert tracer gas due to its heavy molar mass, and based upon previous simulation data from Dutrieue et al suggesting that phase III slope sensitivity to SF6 is maximal at the level of the alveolar duct (generations 20-21).
Participants wore a nose clip and breathed an air mixture containing 0.2% SF₆, while respiratory flows and exhaled breath SF₆ concentrations were monitored by an Innocor photoacoustic gas analyser (Innovision A/S, Odense, Denmark). Participants maintained a steady respiratory rate of approximately 12 breaths per minute and a constant tidal volume of 1L throughout the test, using a real-time visual display of inspired volume as a guide. Once inhaled and exhaled SF₆ concentrations had equalised, participants were switched to breathing room air during an expiration. The test was terminated when the end-tidal concentration of SF₆ in exhaled breath fell below 1/40th of the original concentration for three consecutive breaths. Lung clearance index [10], S_{cond} and S_{acin} [15] were calculated using custom software written with TestPoint (Measurement Computing Corporation, Norton, Massachusetts, USA).

³He-MR was performed using a 0.15 T permanent magnet system (Intermagnetics General Corporation, New York, NY) and a Surrey Medical Imaging Systems console (Surrey, UK). Participants were scanned in the supine position, and inhaled 600ml of a ³He/⁴He mixture from functional residual capacity (FRC), followed by a breath-hold lasting between 2 and 10 seconds, depending upon the pulse sequence being performed. Intermediate-timescale ADC (13ms) was measured using a diffusion-weighted Carr-Purcell-Meiboom-Gill technique [42, 43], and long-timescale ADC (1s) was measured using a stimulated echo sequence [44]. The first seven patients with asthma and the first two healthy controls 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 ADC have been previously reported, with a strong positive correlation observed between the degree of lung inflation and 13ms ADC [43]. In order to aid the interpretation of our results, we also investigated the
relationship between lung volume and long-timescale ADC, in three healthy control subjects and three patients with asthma. 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 using body plethysmography.

A subset of patients with asthma (n = 27) were further characterised using quantitative computed tomography (CT) densitometry. Volumetric whole lung scans were obtained using a Siemens Sensation 16 scanner using the following low dose protocol; 16 x 0.75 mm collimation, 1.5 mm pitch, 120 kVp, 40 mAs, 0.5 seconds rotation time and scanning field of view of 500 mm, 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 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 ratio of mean lung density on expiration to inspiration (MLD E/I), a marker of expiratory air trapping [45], and the fifteenth lower percentile of the inspiratory lung attenuation curve (P15), a marker of emphysema [46].

Statistical analyses were performed using SPSS 20 (IBM Corporation, Somers, New York, USA) and Prism 6 (GraphPad Software Inc., La Jolla, California, USA). Group comparisons were performed using the Student’s t test, one-way analysis of variance with Tukey test for multiple comparisons, or the Mann-Whitney U test for continuous variables, and Fisher’s exact test or the Chi-squared test for proportions. Relationships between continuous variables were investigated using Pearson’s correlation coefficient. A receiver operating characteristic (ROC) curve and a logistic regression model were utilised to determine the relationship between S_acin and the presence or absence of refractory asthma, according to the ATS criteria.
The logistic regression model had the presence or absence of refractory asthma as the dependent variable, and utilized a forward conditional algorithm (SPSS 20) to determine the independent variables entered into the model, from a pool of: S_{acinh}, age, sex, duration of asthma and body mass index (BMI). Previous data on the group standard deviation of ADC at 1s was not available for use in a sample size calculation. However, Wang et al [20] reported a 0.0051 cm^2s^{-1} difference in mean ADC at 1.5s between healthy and asthma groups, with a group standard deviation of 0.0026 cm^2s^{-1} in the healthy group and 0.0055 cm^2s^{-1} in the asthma group, using similar methodology to our own. We calculated that to detect this difference between healthy and asthma groups at 90% power, using a t test with a 5% significance level, we would require 15 patients in each group.

Results

Asthma patient-reported and clinical outcomes in patients with an elevated S_{acinh}

Table 1 shows the demographic and clinical characteristics of the participant groups. Patients with asthma were divided into S_{acinh}-normal and S_{acinh}-high groups, with the upper limit of normal for S_{acinh} being defined as the mean + 1.64 standard deviations in the age-matched control group (0.204 L^{-1}). The three groups were well-matched for age and sex. The S_{acinh}-high group had evidence of suboptimal asthma control, with significantly higher ACQ-6 scores compared to the S_{acinh}-low group. In addition 76% of the S_{acinh}-high group had evidence of refractory asthma (p<0.05 vs S_{acinh} normal asthma) according to the ATS criteria [47], with the majority (n=16/17) having GINA treatment step 4-5 asthma [48]. In contrast 45% of the S_{acinh}-normal group had refractory disease, with patients belonging to the full spectrum of GINA treatment steps. A ROC curve showing the ability of S_{acinh} to distinguish between...
refractory and non-refractory asthma is shown in Figure 2. The area under the ROC curve was 0.77 (95% confidence interval 0.61—0.92; p < 0.01). In contrast, S_acin was far less discriminatory in distinguishing between refractory and non-refractory asthma, with an area under the ROC curve of 0.63 (95% confidence interval 0.44—0.83; p > 0.05). A logistic regression model identified that S_acin was a significant predictor of refractory asthma (p < 0.01), even taking into account the influence of age and sex. These observations were present despite similar levels of eosinophilic airway inflammation in both groups.

**Physiological phenotyping of asthmatics with an elevated S_acin**

Table 2 shows physiological 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. FEV₁ (% pred.) was significantly lower in the S_acin-high group compared to the S_acin-normal group (69.3 vs 90.9, p < 0.01), and the ratio of residual volume to total lung capacity (RV/TLC) was significantly higher (48.3% vs 38.2%, p < 0.01), as was the FRC (% pred.) (131.5% vs 103.7%, p < 0.01). Carbon monoxide transfer coefficient (Kco) did not differ significantly between the groups.

**Imaging-based phenotyping of asthmatics with an elevated S_acin**

Figure 3 shows the CT densitometry data in the two asthma groups. There was evidence of expiratory air trapping in the S_acin-high group, with a significantly raised MLD E/I compared to the S_acin-normal group (0.89 vs 0.83, p < 0.05). However, the inspiratory P₁₅ did not differ between the groups, suggesting that a raised S_acin is not associated with CT density-based assessments of emphysema in patients with asthma. Figure 4 shows the intermediate and long-timescale ADC measurements across the three groups. ADC at 1s was significantly higher in the S_acin-high group compared to the healthy control group (0.024 vs 0.017, p <
0.05), with a trend towards a significant difference between the S\textsubscript{acin}-high and S\textsubscript{acin}-normal asthma groups (0.024 vs 0.019, p = 0.09). There was no evidence that acinar airway disease was attenuated by systemic corticosteroid therapy. In particular, mean S\textsubscript{acin} was 0.256 L\textsuperscript{-1} in patients taking long-term oral corticosteroids (OCS) compared to 0.191 L\textsuperscript{-1} in those not taking OCS (p > 0.05). Mean ADC at 13 ms was 0.121 cm\textsuperscript{2}s\textsuperscript{-1} in patients taking OCS and 0.131 cm\textsuperscript{2}s\textsuperscript{-1} in patients not taking OCS (p > 0.05), while mean ADC at 1s was 0.023 cm\textsuperscript{2}s\textsuperscript{-1} and 0.021 cm\textsuperscript{2}s\textsuperscript{-1} respectively (p > 0.05).

**Evaluation of the contribution of lung volume to apparent diffusion coefficients**

Figure 5-4 shows correlations between ADCs and S\textsubscript{acin} (Panels A and B), FRC (% pred.) (Panels C and D) and MLD E/I (Panels E and F) in patients with asthma. S\textsubscript{acin} correlated weakly with ADC at 13ms (R = 0.38, p < 0.05), but strongly with ADC at 1s (R = 0.65, p < 0.001). S\textsubscript{cond} did not correlate significantly with ADC at either 13ms (R = -0.037, p > 0.05) or 1s (R = 0.101, p > 0.05), indicating that ADC is related specifically to the acinar component of ventilation heterogeneity.

ADC at both 13ms and 1s correlated strongly with the functional residual capacity percent predicted (R = 0.73, p < 0.0001 for ADC at 13ms; R = 0.68, p < 0.0001 for ADC at 1s) and with the mean lung density expiratory / inspiratory ratio, a CT marker of expiratory air trapping (R = 0.77, p < 0.0001 for ADC at 13ms; R = 0.72, p < 0.0001 for ADC at 1s). However, in healthy subjects there were no significant correlations between ADC at 13ms / 1s and either S\textsubscript{acin} or FRC (% pred.).

Figure 6-5 shows the relationship between lung inflation and ADC at 1s in three healthy volunteers (Panel A) and three patients with asthma (Panel B). The correlation was positive
but weak in both cases, only reaching statistical significance in the patients with asthma (p < 0.05). The slope of the lines was shallow, with a 50% increase in lung inflation resulting in a 3.7% increase in ADC in healthy volunteers, and a 4.5% increase in patients with asthma.

Discussion

The main finding of this study is that in patients with asthma, the MBW parameter $S_{acin}$ using the tracer gas SF$_6$ is strongly associated with elevations in long-timescale ADC. However, this association is not observed in healthy subjects. Moreover, elevations in long-timescale ADC cannot be reproduced purely by lung inflation, suggesting that such elevations result from a specific structural abnormality in the pulmonary acinus in patients with asthma.

A number of previous studies have investigated the clinical significance of the acinar lesion in asthma. Farah et al found that improvements in $S_{acin}$ were independently associated with improvements in five-point ACQ score following the initiation of ICS treatment [49], and that markers of ventilation heterogeneity could predict the response to inhaled corticosteroid dose titration [50]. Thompson et al found that $S_{acin}$ correlated with asthma severity, as measured using the Global Initiative for Asthma treatment steps, and that asthma exacerbations were associated with increases in $S_{acin}$ [51]. We observed in the present study that an elevated $S_{acin}$ was present primarily in severe (GINA 4–5) asthma and in 75% of cases refractory asthma, a significantly increased risk of refractory asthma. These observations may reflect a higher prevalence of severe and refractory asthma in our study population; however, the proportion of patient with refractory asthma was significantly higher in Sacin high asthma when compared to Sacin normal asthma. In contrast, we failed to find a similar association
with the conductive ventilation heterogeneity marker $S_{\text{cond}}$. These observations may suggesting that the acinar abnormality captured with SF$_6$ gas washout in asthma may be refractory to conventional pharmacotherapy. In support of this hypothesis a recent interventional study in which switching standard ICS to small particle ICS had no significant effect on $S_{\text{acin}}$ in patients with asthma [52]. However large and appropriately powered intervention studies would be required to confirm this hypothesis fully. In particular, there was no evidence that the acinar lesion was attenuated by systemic corticosteroids, suggesting that it may be underpinned by mechanisms other than eosinophilic airway inflammation. This is supported by a recent interventional study in which switching standard ICS to small particle ICS had no significant effect on $S_{\text{acin}}$ in patients with asthma [52]. In contrast, we found that the association between refractory asthma and the conductive airway disease marker $S_{\text{cond}}$ was far weaker, suggesting that disease in the conducting airways is generally amenable to treatment with standard inhaled corticosteroids, and is less likely to result in severe asthma that is refractory to therapy.

The acinar airways form an asymmetrically dichotomous branching network in three-dimensional space that may be described in terms of its mean airway radius, branch length and branch angle. In this study, we performed MBW using the tracer gas SF$_6$ due to its large molar mass [39], thus increasing the likelihood of probing the pulmonary acinus. Modelling studies by Dutrieue et al have indicated that the convection-diffusion front with SF$_6$ is likely to occur at the level of the alveolar duct (generations 20-21) [41]. 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 13ms, $^3$He ADC is more sensitive to the airway radius than the airway branch length. Long-timescale ADC is a measure of the network properties of the acinar airways, with higher values being associated
with a greater number of inter- and intra-acinar connections. Simulations of long-timescale
ADC 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 values of simulated long-timescale ADC [54]. An increase in airway
branch length causes elevation of long-timescale ADC since it allows greater longitudinal
displacement of helium atoms along the airway axes. Long-timescale ADC may 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 ADC [55].

An important question to address is whether the correlation between $S_{acin}$ and long-timescale
ADC 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 utilised $^3$He MR lung morphometry to assess the changes that occur in the
acinar airways during lung inflation in healthy subjects [56]. They concluded that lung
inflation occurs primarily by alveolar recruitment, and to a lesser extent by the expansion of
alveolar ducts. The alveolar sleeve width actually decreased with increasing lung inflation.
The expansion of alveolar ducts would be expected to increase short or intermediate-
timescale ADC, and indeed it is known that 13ms 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 ADC, suggesting that hyperinflation alone cannot account for the
strong association between $S_{acin}$ and long-timescale ADC.
We observed strong correlations between the CT marker of expiratory air trapping MLD E/I and both intermediate and long-timescale ADC, suggesting that there may 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 may be micro-CT of surgical lung biopsies or resected lung specimens, as has been performed in patients with COPD [57]. We found no evidence of emphysema in patients with asthma and a raised S_{acim} with neither P_{15} nor KCO differing between the S_{acim}-normal and S_{acim}-high groups. Verbanck et al also observed normal KCO values in patients with asthma [16], 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 asthma [59,60], and autopsy studies have suggested that this may be due to a subtle breakdown of lung architecture [61,62].

Our study has a number of potential limitations. Firstly patients were drawn predominantly from a secondary care centre and as such the results may not be generalisable to an unselected asthma population. In particular, our study group was likely to have a higher proportion of patients with refractory asthma and those taking long-term oral corticosteroids than a general asthma population. However it is important to note that the rationale for referral to our asthma centre and difficult asthma clinic included optimisation of other factors such as treatment non-adherence, rhino sinusitis and psychological/behavioural issues. As such the selected population was not specifically identified from a treatment refractory cohort.
Secondly the mean age of patients in our study was 57.4 years, which may be slightly older than an unselected asthma population, and the proportion of males among our asthma group was 51.4%, compared to 36.9% in a previously published large difficult asthma cohort [63]. In order to mitigate these effects, we controlled for both age and sex in our logistic regression models.

Thirdly it has previously been shown that the pulmonary acinus may be sensitive to cigarette smoking [64]. Therefore, despite the fact that ‘real world’ asthma populations include both ex-smokers and current smokers, we chose to recruit only never-smokers or ex-smokers with a smoking history (< 10 pack years) to this study. This was done deliberately as we wanted to explore the relationship between a non-invasive marker of small airway dysfunction and airway diffusion measurements in asthma and our measurements would have been confounded by smoking associated acinar changes if we had selected smoking, heavy ex-smoking populations. However it remains possible that differences in low-grade cigarette smoke exposure may have accounted for some of the proposed structural changes observed within the acinus in the Sacin high population.

Finally a further potential limitation of the study was that the MR pulse sequences used did not provide three-dimensional spatial information. Future studies incorporating three-dimensional spatial encoding of ADC may 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 patients with asthma, causing subtle alterations in diffusion within the acinar airways. In addition the proportion of cases with refractory asthma...
and a high \( S_{\text{acin}} \) was significantly associated with refractory asthma greater when compared to patients with a normal \( S_{\text{acin}} \), suggesting that the lesion may be clinically important. However, this latter observation must be regarded exploratory and warrants further prospective evaluation.

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


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Table 1: Demographic and clinical characteristics of participant groups

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 17)</th>
<th>Asthma S_{acis} normal (n = 20)</th>
<th>Asthma S_{acis} high (n = 17)</th>
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<tr>
<td>Age (years)</td>
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<td>54.2 (3.1)</td>
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<td>Height (cm)</td>
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<td>78.1 (3.3)</td>
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<td>28.9 (1.3)</td>
<td>31.2 (1.4)**</td>
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<td>Smoking status</td>
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<td>Never smokers (n [%])</td>
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<td>15 (75%)</td>
<td>10 (59%)</td>
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<td>0 (0 – 8)</td>
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<td>Duration of asthma (years)</td>
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<tr>
<td>ACQ-6 score*</td>
<td>-</td>
<td>1.43 (0.26)</td>
<td>2.14 (0.22)</td>
</tr>
<tr>
<td>AQLQ(S) score†</td>
<td>-</td>
<td>5.61 (0.23)</td>
<td>4.95 (0.31)</td>
</tr>
<tr>
<td>Sputum neutrophil count (%)</td>
<td>-</td>
<td>57.2 (6.0)</td>
<td>61.8 (7.1)</td>
</tr>
<tr>
<td>Sputum eosinophil count (%)‡</td>
<td>-</td>
<td>2.69 (1.23 – 5.89)</td>
<td>1.76 (0.76 – 4.04)</td>
</tr>
<tr>
<td>Blood eosinophil count (×10⁹/L)</td>
<td>-</td>
<td>0.33 (0.04)</td>
<td>0.34 (0.07)</td>
</tr>
<tr>
<td>Daily dose of inhaled corticosteroid (beclometasone dipropionate equivalent [μg])</td>
<td>-</td>
<td>1000 – 2000</td>
<td>1600 – 2000</td>
</tr>
<tr>
<td>Use of long-acting beta-agonists (% of subjects)</td>
<td>-</td>
<td>75</td>
<td>94</td>
</tr>
<tr>
<td>Regular use of oral prednisolone (% of subjects)</td>
<td>-</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>Use of leukotriene receptor antagonist (% of subjects)</td>
<td>-</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>Use of a methylxanthine (% of subjects)§</td>
<td>-</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>Refractory asthma (% positive)¶</td>
<td>-</td>
<td>45</td>
<td>76</td>
</tr>
</tbody>
</table>

ACQ-6 = six-point Asthma Control Questionnaire; AQLQ(S) = standardised Asthma Quality of Life Questionnaire.

† Expressed as geometric mean (95% confidence interval). Log-transformed data compared between groups using Student’s t test.

‡ As defined by the Global Initiative for Asthma [48]. Expressed as number of patients receiving treatment at step 2: step 3: step 4: step 5.

¶ Refractory asthma defined according to the American Thoracic Society Workshop definition [47].

Data expressed as mean (standard error) or proportions, unless stated otherwise. Groups compared using one-way analysis of variance with Tukey test for multiple comparisons or Student’s t test for
parametric data, Mann-Whitney U test for non-parametric data, and Chi-squared test or Fisher’s exact test for proportions. Significant differences across or between groups denoted * (p < 0.05) with trends towards significance denoted ¥ (p < 0.1). Significant differences compared to healthy control group denoted ¥¥ (p < 0.01) or ¥¥¥ (p < 0.001).

Table 2: Physiological, computed tomography and magnetic resonance data across participant groups

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 17)</th>
<th>Asthma S_{a}in normal (n = 20)</th>
<th>Asthma S_{a}in high (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ (% pred.)****</td>
<td>97.7 (3.4)</td>
<td>83.9 (3.8)¥</td>
<td>65.4 (4.8)¥¥</td>
</tr>
<tr>
<td>FEV₁ z score****</td>
<td>-0.18 (0.23)</td>
<td>-1.16 (0.26)¥</td>
<td>-2.28 (0.29)¥</td>
</tr>
<tr>
<td>FVC (% pred.)**</td>
<td>107.7 (3.7)</td>
<td>94.4 (2.9)¥</td>
<td>91.6 (3.3)¥</td>
</tr>
<tr>
<td>FVC z score**</td>
<td>0.53 (0.27)</td>
<td>-0.42 (0.21)¥</td>
<td>-0.66 (0.26)¥¥</td>
</tr>
<tr>
<td>FEV₁/FVC (%)****</td>
<td>72.1 (1.7)</td>
<td>70.6 (2.5)¥</td>
<td>55.6 (3.3)¥¥</td>
</tr>
<tr>
<td>FEV₁/FVC z score***</td>
<td>-1.03 (0.23)</td>
<td>-1.23 (0.30)</td>
<td>-2.73 (0.31)¥¥</td>
</tr>
<tr>
<td>FRC (L)**</td>
<td>3.67 (0.26)</td>
<td>3.08 (0.23)</td>
<td>4.28 (0.26)¥</td>
</tr>
<tr>
<td>FRC (% pred.)*</td>
<td>114.4 (6.2)</td>
<td>103.7 (6.7)</td>
<td>131.5 (6.2)¥¥</td>
</tr>
<tr>
<td>TLC (L)</td>
<td>6.92 (0.48)</td>
<td>5.70 (0.34)</td>
<td>6.77 (0.39)</td>
</tr>
<tr>
<td>TLC (% pred.)</td>
<td>109.8 (3.7)</td>
<td>103.3 (3.8)</td>
<td>109.5 (3.4)</td>
</tr>
<tr>
<td>RV/TLC (%)****</td>
<td>31.9 (2.2)</td>
<td>38.2 (1.9)¥</td>
<td>48.3 (2.3)¥¥</td>
</tr>
<tr>
<td>RV/TLC (% pred.)****</td>
<td>88.3 (3.6)</td>
<td>104.8 (4.2)</td>
<td>125.6 (6.0)¥¥</td>
</tr>
<tr>
<td>VA/TLC (%)****</td>
<td>88.2 (1.8)</td>
<td>82.0 (1.9)</td>
<td>74.3 (1.9)¥¥</td>
</tr>
</tbody>
</table>
FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; FRC = functional residual capacity from body plethysmography; TLC = total lung capacity; RV = residual volume; VA = alveolar volume from single breath helium dilution; KCO = carbon monoxide transfer coefficient; LCI = lung clearance index; MLD E/I = mean lung density expiratory / inspiratory ratio; P15 = Fifteenth lower percentile of inspiratory lung attenuation curve; ADC = apparent diffusion coefficient.

Data expressed as mean (standard error). Groups compared using one-way analysis of variance with Tukey test for multiple comparisons or Student’s t test. Significant differences across groups denoted *(p < 0.05), **(p < 0.01), ****(p < 0.001) or *****(p < 0.0001). Significant differences compared to healthy control group denoted #*(p < 0.05), ##*(p < 0.01), ###*(p < 0.001) or ####*(p < 0.0001). Significant differences between asthma Sacin-low and Sacin-high groups denoted ¥*(p < 0.05), ¥¥*(p < 0.01), ¥¥¥*(p < 0.001) or ¥¥¥¥*(p < 0.0001).
Figure legends

Figure 1: Schematic diagram of length scales probed by helium apparent diffusion coefficients
Dotted line represents maximal anatomical sensitivity of SF₆ phase III slopes [41].

Figure 2: Receiver operating characteristic curve showing the discriminatory ability of $S_{\text{cin}}$ for distinguishing refractory and non-refractory asthma

Figure 3: Quantitative computed tomography densitometry between asthma groups
Panels A and B display the quantitative assessment of expiratory air trapping in patients with low and high levels of air trapping, respectively. Coloured spheres represent areas of lung > 1 ml in volume with an attenuation on expiratory scans of < -856 Hounsfield units. Panels C and D display the ratio of mean lung density on expiration to inspiration (MLD E/I), and the fifteenth lower percentile of the inspiratory lung attenuation curve (P₁₅), respectively, in patients with asthma. Error bars indicate means ± standard errors of the mean.

Figure 4: Apparent diffusion coefficients (ADC) across groups
ADC at 13ms (Panel A) and 1s (Panel B) are shown across healthy and asthma groups. Error bars indicate means ± standard errors of the mean.

Figure 54: Correlations between $^3$He-MR, CT and physiological variables in patients with asthma

Correlations are shown between apparent diffusion coefficients and $S_{acin}$ (Panels A and B), functional residual capacity percent predicted (Panels C and D), and the ratio of mean lung density on expiration to inspiration (Panels E and F). Best-fit linear regression lines and Pearson correlation coefficients are shown.

Figure 65: Change in apparent diffusion coefficient (%) against change in volume of gas in the lungs (%) in healthy subjects and patients with asthma

Correlations are shown between percentage change in ADC and percentage change in volume of gas in the lungs in three healthy subjects (Panel A) and three patients with asthma (Panel B). The three participants in each case are denoted with different symbols. $V = $ volume of gas in lungs; $V_{FRC} =$ volume of gas in lungs at functional residual capacity; $ADC_v =$ ADC at lung volume $V$; $ADC_{FRC} =$ ADC at functional residual capacity (extrapolated).
Characterisation of Acinar Airspace Involvement in Asthma using Inert Gas Washout and Hyperpolarised $^3$Helium Magnetic Resonance

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Abstract

Background
The multiple breath washout (MBW) parameter $S_{acin}$ is thought to be a marker of acinar airway involvement, but has not been validated using quantitative imaging techniques in asthma.

Objective
We aimed to utilise $^3$He diffusion magnetic resonance ($^3$He-MR) at multiple diffusion timescales and quantitative computed tomography (CT) densitometry to determine the nature of acinar airway involvement in asthma.

Methods
Thirty-seven patients with asthma and seventeen age-matched healthy controls underwent spirometry, body plethysmography, MBW (using the tracer gas sulphur hexafluoride) and $^3$He-MR. A subset of patients with asthma ($n = 27$) underwent quantitative CT densitometry.

Results
Ninety-four percent (16/17) of patients with an elevated $S_{acin}$ had GINA treatment step 4/5 asthma and 13/17 had refractory disease. The apparent diffusion coefficient (ADC) of $^3$He at 1s was significantly higher in patients with $S_{acin}$-high asthma compared to healthy controls (0.024 vs 0.017, $p < 0.05$). $S_{acin}$ correlated strongly with ADC at 1s ($R = 0.65$, $p < 0.001$), but weakly with ADC at 13ms ($R = 0.38$, $p < 0.05$). ADC at both 13ms and 1s correlated strongly with the mean lung density expiratory / inspiratory ratio, a CT marker of expiratory air trapping ($R = 0.77$, $p < 0.0001$ for ADC at 13ms; $R = 0.72$, $p < 0.001$ for ADC at 1s).
Conclusion

$S_{acini}$ is associated with alterations in long-range diffusion within the acinar airways and gas trapping. The precise anatomical nature and mechanistic role in severe asthma requires further evaluation.

Key words: Asthma, small airways, acinus, physiology
Clinical implications:

There is evidence of a structural abnormality in the pulmonary acinus in patients with asthma, which is present primarily in severe disease.

Capsule summary:

Quantitative imaging techniques are utilised to determine the structural correlates of $S_{acin}$, a putative physiological marker of acinar airway disease. The results suggest a structural abnormality in this region which warrants further clinico-pathological evaluation.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^3$He-MR</td>
<td>Hyperpolarised $^3$helium diffusion magnetic resonance</td>
</tr>
<tr>
<td>ACQ</td>
<td>Asthma Control Questionnaire</td>
</tr>
<tr>
<td>ADC</td>
<td>Apparent diffusion coefficient</td>
</tr>
<tr>
<td>AQLQ(S)</td>
<td>Standardised Asthma Quality of Life Questionnaire</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CDI</td>
<td>Convection-dependent inhomogeneity</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DCDI</td>
<td>Diffusion-convection-dependent inhomogeneity</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional residual capacity</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled corticosteroids</td>
</tr>
<tr>
<td>KCO</td>
<td>Carbon monoxide transfer coefficient</td>
</tr>
<tr>
<td>MBW</td>
<td>Multiple breath inert gas washout</td>
</tr>
<tr>
<td>MLD E/I</td>
<td>Mean lung density expiratory / inspiratory ratio</td>
</tr>
<tr>
<td>$P_{15}$</td>
<td>Fifteenth lower percentile of the inspiratory lung attenuation curve</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operating characteristic</td>
</tr>
<tr>
<td>RV</td>
<td>Residual volume</td>
</tr>
<tr>
<td>$S_{\text{acin}}$</td>
<td>Acinar ventilation heterogeneity</td>
</tr>
<tr>
<td>$S_{\text{cond}}$</td>
<td>Conductive ventilation heterogeneity</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
</tbody>
</table>
Introduction

Asthma is a chronic inflammatory airway disease that is characterised by variable airflow obstruction, airway hyperresponsiveness and structural remodelling in both the large and small airways [1]. Understanding the site and nature of small airways disease in asthma is important as it may allow the development of therapies that target this region of the lung or better application of existing therapies such as extra fine particle inhalers [2].

While it is known that inflammatory and structural changes in asthma 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 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 non-invasively probe the structure of the acinar airways in patients with asthma. These include the physiological assessment of gas mixing using multiple breath inert gas washout (MBW) [10], measurement of gas diffusion using hyperpolarised noble gas magnetic resonance techniques [11], and computed tomography (CT) densitometry to evaluate expiratory air trapping [12]. However to date there has not been a comprehensive assessment of the acinar airways in asthma using these approaches together.

There are thought to be two independent mechanisms of gas mixing inefficiency in the lungs, namely convection-dependent inhomogeneity (CDI) and diffusion-convection-dependent inhomogeneity (DCDI) [13,14]. CDI arises due to unequal convective ventilation between relatively large lung units subtended by conducting airways. DCDI is a more complex mechanism that occurs due to 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 magnitude. The MBW parameters $S_{\text{cond}}$ and $S_{\text{acin}}$ were proposed by Verbanck et al. as measures of CDI and DCDI, respectively [15]. Since in health, the convection-diffusion front is thought to be located within the pulmonary acinus, $S_{\text{acin}}$ was proposed as a putative physiological marker of acinar airspace disease. However, the precise location of the convection-diffusion front is heavily dependent upon the molar mass of the inert tracer gas being used, with heavier gases such as sulphur hexafluoride (SF$_6$) probing more distal regions of the pulmonary acinus than lighter gases such as N$_2$ [10]. Elevations in $S_{\text{acin}}$ have been observed in patients with asthma, leading to the suggestion that this condition is characterised by a specific structural abnormality in the pulmonary acinus [16]. However, the precise nature of this structural abnormality has not been elucidated.

Hyperpolarised $^3$helium diffusion magnetic resonance ($^3$He-MR) is a technique that allows microstructural changes at the level of alveoli and acinar airways to be examined non-invasively, under resting physiological conditions [11]. The apparent diffusion coefficient (ADC) of $^3$He within the pulmonary acinus may be measured across a wide range of timescales, from 1ms to 10s. Short or intermediate timescales of the order of a few milliseconds correspond to diffusion within a single alveolus or a single acinar airway, respectively, while long timescales of the order of seconds correspond to diffusion within several acinar airways [11], as illustrated in Figure 1. $^3$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-ADC is elevated in both patients with emphysema [17-23], and in animal models of emphysema [24-27], in comparison with values obtained in healthy lungs. Moreover, in a number of these studies ADC was found to correlate with quantitative histological measures of emphysema such as the mean linear intercept, mean
alveolar internal area and mean chord length [20, 22, 24-27]. Air trapping may be assessed
using physiological measurements of lung volumes [28], 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 [29].

We aimed to utilise $^3$He-MR at multiple diffusion timescales and quantitative CT
densitometry to determine the structural correlates of the multiple breath washout marker $S_{acin}$
in asthma, using SF$_6$-MBW. We hypothesised that (i) asthma patients with an elevated $S_{acin}$
would manifest altered long range diffusion suggestive of intra-acinar airway disease, and (ii)
the degree of acinar involvement in asthma would be independent of lung hyperinflation.

**Methods**

Thirty-seven patients with asthma and seventeen age-matched healthy control subjects were
recruited. All of patients within this study were recruited from our secondary care asthma
centre [Glenfield Hospital; Leicester]. The centre primarily evaluates patients at GINA
treatment steps 3-5 to optimise their disease control and any potential comorbidities e.g. rhino
sinusitis, treatment non adherence. 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 asthma population in the UK 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 six weeks. All participants were never
smokers or ex-smokers with less than 10 pack years’ smoking history. Asthma was diagnosed in a secondary care setting according to British Thoracic Society guidelines [30]. The study was approved by the National Research and Ethics Committee – East Midlands, Leicester, and all participants gave their written informed consent.

Patients with asthma completed the six-point Asthma Control Questionnaire (ACQ-6) [31] and the standardised Asthma Quality of Life Questionnaire (AQLQ(S)) [32]. Participants were administered 200 micrograms of salbutamol via a metered-dose inhaler and spacer, to minimise the confounding effects of airway smooth muscle tone on physiological and imaging assessments. Spirometry, body plethysmography and measurement of carbon monoxide diffusing capacity were performed according to American Thoracic Society (ATS) / European Respiratory Society guidelines [33-35]. Predicted values and standardised residuals (z scores) were derived using the Global Lung Function Initiative (2012) equations for spirometry [36], and the European Community for Steel and Coal (1993) equations for lung volumes and carbon monoxide transfer coefficient [37]. Induced sputum inflammatory cell counts were obtained in patients with asthma using a previously published method [38].

MBW was performed according to current guidelines [39] using the SF₆ wash-in method described by Horsley et al [40]. SF₆ was chosen as the inert tracer gas due to its heavy molar mass, and based upon previous simulation data from Dutrieue et al suggesting that phase III slope sensitivity to SF₆ is maximal at the level of the alveolar duct (generations 20-21), Figure 1 [41]. Participants wore a nose clip and breathed an air mixture containing 0.2% SF₆, while respiratory flows and exhaled breath SF₆ concentrations were monitored by an Innocor photoacoustic gas analyser (Innovision A/S, Odense, Denmark). Participants maintained a steady respiratory rate of approximately 12 breaths per minute and a constant tidal volume of
IL throughout the test, using a real-time visual display of inspired volume as a guide. Once inhaled and exhaled SF$_6$ concentrations had equalised, 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 fell below $1/40^{th}$ of the original concentration for three consecutive breaths. Lung clearance index [10], $S_{\text{cond}}$ and $S_{\text{acin}}$ [15] were calculated using custom software written with TestPoint (Measurement Computing Corporation, Norton, Massachusetts, USA).

$^3$He-MR was performed using a 0.15 T permanent magnet system (Intermagnetics General Corporation, New York, NY) and a Surrey Medical Imaging Systems console (Surrey, UK). Participants were scanned in the supine position, and inhaled 600ml of a $^3$He/$^4$He mixture from functional residual capacity (FRC), followed by a breath-hold lasting between 2 and 10 seconds, depending upon the pulse sequence being performed. Intermediate-timescale ADC (13ms) was measured using a diffusion-weighted Carr-Purcell-Meiboom-Gill technique [42, 43], and long-timescale ADC (1s) was measured using a stimulated echo sequence [44]. The first seven patients with asthma and the first two healthy controls 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 ADC have been previously reported, with a strong positive correlation observed between the degree of lung inflation and 13ms ADC [43]. In order to aid the interpretation of our results, we also investigated the relationship between lung volume and long-timescale ADC, in three healthy control subjects and three patients with asthma. 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 using body plethysmography.
A subset of patients with asthma (n = 27) were further characterised using quantitative computed tomography (CT) densitometry. Volumetric whole lung scans were obtained using a Siemens Sensation 16 scanner using the following low dose protocol; 16 x 0.75 mm collimation, 1.5 mm pitch, 120 kVp, 40 mAs, 0.5 seconds rotation time and scanning field of view of 500 mm, 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 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 ratio of mean lung density on expiration to inspiration (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 using SPSS 20 (IBM Corporation, Somers, New York, USA) and Prism 6 (GraphPad Software Inc., La Jolla, California, USA). Group comparisons were performed using the Student’s t test, one-way analysis of variance with Tukey test for multiple comparisons, or the Mann-Whitney U test for continuous variables, and Fisher’s exact test or the Chi-squared test for proportions. Relationships between continuous variables were investigated using Pearson’s correlation coefficient. Previous data on the group standard deviation of ADC at 1s was not available for use in a sample size calculation. However, Wang et al [20] reported a 0.0051 cm²s⁻¹ difference in mean ADC at 1.5s between healthy and asthma groups, with a group standard deviation of 0.0026 cm²s⁻¹ in the healthy group and 0.0055 cm²s⁻¹ in the asthma group, using similar methodology to our own. We calculated that to detect this difference between healthy and asthma groups at 90% power, using a t test with a 5% significance level, we would require 15 patients in each group.
Results

Asthma patient-reported and clinical outcomes in patients with an elevated $S_{acin}$

Table 1 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 standard deviations in the age-matched control group (0.204 L$^{-1}$). The three 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 to the $S_{acin}$-normal group. In addition 76% of the $S_{acin}$-high group had evidence of refractory asthma (p<0.05 vs $S_{acin}$-normal asthma) according to the ATS criteria [47], with the majority (n=16/17) having GINA treatment step 4-5 asthma [48]. In contrast 45% of the $S_{acin}$-normal group had refractory disease, with patients belonging to the full spectrum of GINA treatment steps.

Physiological phenotyping of asthmatics with an elevated $S_{acin}$

Table 2 shows physiological 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$_1$ (% pred.) was significantly lower in the $S_{acin}$-high group compared to the $S_{acin}$-normal group (69.3 vs 90.9, p < 0.01), and the ratio of residual volume to total lung capacity (RV/TLC) was significantly higher (48.3% vs 38.2%, p < 0.01), as was the FRC (% pred.) (131.5% vs 103.7%, p < 0.01). Carbon monoxide transfer coefficient (Kco) did not differ significantly between the groups.
Imaging-based phenotyping of asthmatics with an elevated $S_{\text{acin}}$

Figure 2 shows the CT densitometry data in the two asthma groups. There was evidence of expiratory air trapping in the $S_{\text{acin}}$-high group, with a significantly raised MLD E/I compared to the $S_{\text{acin}}$-normal group (0.89 vs 0.83, $p < 0.05$). However, the inspiratory $P_{15}$ did not differ between the groups, suggesting that a raised $S_{\text{acin}}$ is not associated with CT density-based assessments of emphysema in patients with asthma. Figure 3 shows the intermediate and long-timescale ADC measurements across the three groups. ADC at 1s was significantly higher in the $S_{\text{acin}}$-high group compared to the healthy control group (0.024 vs 0.017, $p < 0.05$), with a trend towards a significant difference between the $S_{\text{acin}}$-high and $S_{\text{acin}}$-normal asthma groups (0.024 vs 0.019, $p = 0.09$). There was no evidence that acinar airway disease was attenuated by systemic corticosteroid therapy. In particular, mean $S_{\text{acin}}$ was 0.256 L$^{-1}$ in patients taking long-term oral corticosteroids (OCS) compared to 0.191 L$^{-1}$ in those not taking OCS ($p > 0.05$). Mean ADC at 13 ms was 0.121 cm$^2$s$^{-1}$ in patients taking OCS and 0.131 cm$^2$s$^{-1}$ in patients not taking OCS ($p > 0.05$), while mean ADC at 1s was 0.023 cm$^2$s$^{-1}$ and 0.021 cm$^2$s$^{-1}$ respectively ($p > 0.05$).

Evaluation of the contribution of lung volume to apparent diffusion coefficients

Figure 4 shows correlations between ADCs and $S_{\text{acin}}$ (Panels A and B), FRC (% pred.) (Panels C and D) and MLD E/I (Panels E and F) in patients with asthma. $S_{\text{acin}}$ correlated weakly with ADC at 13ms ($R = 0.38$, $p < 0.05$), but strongly with ADC at 1s ($R = 0.65$, $p < 0.001$). $S_{\text{cond}}$ did not correlate significantly with ADC at either 13ms ($R = -0.037$, $p > 0.05$) or 1s ($R = 0.101$, $p > 0.05$), indicating that ADC is related specifically to the acinar component of ventilation heterogeneity.
ADC at both 13ms and 1s correlated strongly with the functional residual capacity percent predicted (R = 0.73, p < 0.0001 for ADC at 13ms; R = 0.68, p < 0.0001 for ADC at 1s) and with the mean lung density expiratory / inspiratory ratio, a CT marker of expiratory air trapping (R = 0.77, p < 0.0001 for ADC at 13ms; R = 0.72, p < 0.0001 for ADC at 1s). However, in healthy subjects there were no significant correlations between ADC at 13ms / 1s and either $S_{acin}$ or FRC (% pred.).

**Figure 5** shows the relationship between lung inflation and ADC at 1s in three healthy volunteers (Panel A) and three patients with asthma (Panel B). The correlation was positive but weak in both cases, only reaching statistical significance in the patients with asthma (p < 0.05). The slope of the lines was shallow, with a 50% increase in lung inflation resulting in a 3.7% increase in ADC in healthy volunteers, and a 4.5% increase in patients with asthma.

**Discussion**

The main finding of this study is that in patients with asthma, the MBW parameter $S_{acin}$ using the tracer gas SF$_6$ is strongly associated with elevations in long-timescale ADC. However, this association is not observed in healthy subjects. Moreover, elevations in long-timescale ADC cannot be reproduced purely by lung inflation, suggesting that such elevations result from a specific structural abnormality in the pulmonary acinus in patients with asthma.

A number of previous studies have investigated the clinical significance of the acinar lesion in asthma. Farah *et al* found that improvements in $S_{acin}$ were independently associated with improvements in five-point ACQ score following the initiation of ICS treatment [49], and
that markers of ventilation heterogeneity could predict the response to inhaled corticosteroid
dose titration [50]. Thompson et al found that \( S_{\text{acin}} \) correlated with asthma severity, as
measured using the Global Initiative for Asthma treatment steps, and that asthma
exacerbations were associated with increases in \( S_{\text{acin}} \) [51]. We observed in the present study
that an elevated \( S_{\text{acin}} \) was present primarily in severe (GINA 4-5) asthma and in
approximately 75% of cases refractory asthma. These observations may reflect a higher
prevalence of severe and refractory asthma in our study population; however the proportion
of patient with refractory asthma was significantly higher in \( S_{\text{acin}} \)-high asthma when
compared to \( S_{\text{acin}} \)-normal asthma. In contrast, we failed to find a similar association with the
conductive ventilation heterogeneity marker \( S_{\text{cond}} \). These observations may suggest that the
acinar abnormality captured with SF\(_6\) gas washout in asthma may be refractory to
conventional pharmacotherapy. In support of this hypothesis a recent interventional study in
which switching standard ICS to small particle ICS had no significant effect on \( S_{\text{acin}} \) in
patients with asthma [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 three-
dimensional space that may be described in terms of its mean airway radius, branch length
and branch angle. In this study, we performed MBW using the tracer gas SF\(_6\) due to its large
molar mass [39], thus increasing the likelihood of probing the pulmonary acinus. Modelling
studies by Dutrieue et al have indicated that the convection-diffusion front with SF\(_6\) is likely
to occur at the level of the alveolar duct (generations 20-21) [41]. 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 13ms, \(^3\)He ADC is more
sensitive to the airway radius than the airway branch length. Long-timescale ADC is a
measure of the network properties of the acinar airways, with higher values being associated
with a greater number of inter- and intra-acinar connections. Simulations of long-timescale
ADC 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 values of simulated long-timescale ADC [54]. An increase in airway
branch length causes elevation of long-timescale ADC since it allows greater longitudinal
displacement of helium atoms along the airway axes. Long-timescale ADC may 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 ADC [55].

An important question to address is whether the correlation between $S_{\text{acin}}$ and long-timescale
ADC 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
$S_{\text{acin}}$. Hajari et al utilised $^3$He MR lung morphometry to assess the changes that occur in the
acinar airways during lung inflation in healthy subjects [56]. They concluded that lung
inflation occurs primarily by alveolar recruitment, and to a lesser extent by the expansion of
alveolar ducts. The alveolar sleeve width actually decreased with increasing lung inflation.
The expansion of alveolar ducts would be expected to increase short or intermediate-
timescale ADC, and indeed it is known that 13ms 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 ADC, suggesting that hyperinflation alone cannot account for the
strong association between $S_{\text{acin}}$ and long-timescale ADC.
We observed strong correlations between the CT marker of expiratory air trapping MLD E/I and both intermediate and long-timescale ADC, suggesting that there may 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 may be micro-CT of surgical lung biopsies or resected lung specimens, as has been performed in patients with COPD [57]. We found no evidence of emphysema in patients with asthma and a raised $S_{\text{acin}}$, with neither $P_{15}$ nor $K_{\text{CO}}$ differing between the $S_{\text{acin}}$-normal and $S_{\text{acin}}$-high groups. Verbanck et al also observed normal $K_{\text{CO}}$ values in patients with asthma [16], 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 asthma [59,60], and autopsy studies have suggested that this may be due to a subtle breakdown of lung architecture [61,62].

Our study has a number of potential limitations. Firstly patients were drawn predominantly from a secondary care centre and as such the results may not be generalisable to an unselected asthma population. In particular, our study group was likely to have a higher proportion of patients with refractory asthma and those taking long-term oral corticosteroids than a general asthma population. However it is important to note that the rationale for referral to our asthma centre and difficult asthma clinic included optimisation of other factors such as treatment non-adherence, rhino sinusitis and psychological/behavioural issues. As such the selected population was not specifically identified from a treatment refractory cohort.
Secondly the mean age of patients in our study was 57.4 years, which may be slightly older than an unselected asthma population, and the proportion of males among our asthma group was 51.4%, compared to 36.9% in a previously published large difficult asthma cohort [63]. In order to mitigate these effects, we controlled for both age and sex in our logistic regression models.

Thirdly it has previously been shown that the pulmonary acinus may be sensitive to cigarette smoking [64]. Therefore, despite the fact that ‘real world’ asthma populations include both ex-smokers and current smokers, we chose to recruit only never-smokers or ex-smokers with a smoking history (< 10 pack years) to this study. This was done deliberately as we wanted to explore the relationship between a non-invasive marker of small airway dysfunction and airway diffusion measurements in asthma and our measurements would have been confounded by smoking associated acinar changes if we had selected smoking, heavy ex-smoking populations. However it remains possible that differences in low-grade cigarette smoke exposure may have accounted for some of the proposed structural changes observed within the acinus in the Sacin high population.

Finally a further potential limitation of the study was that the MR pulse sequences used did not provide three-dimensional spatial information. Future studies incorporating three-dimensional spatial encoding of ADC may 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 patients with asthma, causing subtle alterations in diffusion within the acinar airways. In addition the proportion of cases with refractory asthma
and a high $S_{\text{acin}}$ was significantly greater when compared to patients with a normal $S_{\text{acin}}$, suggesting that the lesion may be clinically important. However this latter observation must be regarded exploratory and warrants further prospective evaluation.

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


Table 1: Demographic and clinical characteristics of participant groups

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 17)</th>
<th>Asthma $S_{acini}$ normal (n = 20)</th>
<th>Asthma $S_{acini}$ high (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.4 (3.3)</td>
<td>54.2 (3.1)</td>
<td>61.2 (1.9)</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>47</td>
<td>40</td>
<td>65</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.6 (2.6)</td>
<td>164.8 (2.5)</td>
<td>169.7 (1.9)</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>75.0 (2.7)</td>
<td>78.1 (3.3)</td>
<td>90.4 (5.0)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)*</td>
<td>25.8 (0.8)</td>
<td>28.9 (1.3)</td>
<td>31.2 (1.4)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smokers (n [%])</td>
<td>15 (88%)</td>
<td>15 (75%)</td>
<td>10 (59%)</td>
</tr>
<tr>
<td>Ex-smokers (n [%])</td>
<td>2 (12%)</td>
<td>5 (25%)</td>
<td>7 (41%)</td>
</tr>
<tr>
<td>Pack years (median [range])</td>
<td>0 (0 – 2)</td>
<td>0 (0 – 7)</td>
<td>0 (0 – 8)</td>
</tr>
<tr>
<td>Age of onset of asthma symptoms (years)</td>
<td>-</td>
<td>23.4 (5.0)</td>
<td>27.5 (5.3)</td>
</tr>
<tr>
<td>Duration of asthma (years)</td>
<td>-</td>
<td>30.9 (3.8)</td>
<td>33.7 (5.1)</td>
</tr>
<tr>
<td>Atopic status (% positive)</td>
<td>-</td>
<td>85</td>
<td>82</td>
</tr>
<tr>
<td>ACQ-6 score*</td>
<td>-</td>
<td>1.43 (0.26)</td>
<td>2.14 (0.22)</td>
</tr>
<tr>
<td>AQLQ(S) score†</td>
<td>-</td>
<td>5.61 (0.23)</td>
<td>4.95 (0.31)</td>
</tr>
<tr>
<td>Sputum neutrophil count (%)</td>
<td>-</td>
<td>57.2 (6.0)</td>
<td>61.8 (7.1)</td>
</tr>
<tr>
<td>Sputum eosinophil count (%)†</td>
<td>-</td>
<td>2.69 (1.23 – 5.89)</td>
<td>1.76 (0.76 – 4.04)</td>
</tr>
<tr>
<td>Blood eosinophil count ($\times 10^9$/L)</td>
<td>-</td>
<td>0.33 (0.04)</td>
<td>0.34 (0.07)</td>
</tr>
<tr>
<td>Daily dose of inhaled corticosteroid (beclometasone dipropionate equivalent [μg])</td>
<td>-</td>
<td>1000 0 – 2000</td>
<td>1600 200 – 2000</td>
</tr>
<tr>
<td>Use of long-acting beta-agonists (% of subjects)</td>
<td>-</td>
<td>75</td>
<td>94</td>
</tr>
<tr>
<td>Regular use of oral prednisolone (% of subjects)</td>
<td>-</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>Use of leukotriene receptor antagonist (% of subjects)</td>
<td>-</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>Use of a methylxanthine (% of subjects)²</td>
<td>-</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>--------------------------</td>
<td>---</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Refractory asthma (% positive)¶,¶</td>
<td>-</td>
<td>45</td>
<td>76</td>
</tr>
</tbody>
</table>

ACQ-6 = six-point Asthma Control Questionnaire; AQLQ(S) = standardised Asthma Quality of Life Questionnaire.

† Expressed as geometric mean (95% confidence interval). Log-transformed data compared between groups using Student’s $t$ test.

‡ As defined by the Global Initiative for Asthma [48]. Expressed as number of patients receiving treatment at step 2: step 3: step 4: step 5.

¶ Refractory asthma defined according to the American Thoracic Society Workshop definition [47].

Data expressed as mean (standard error) or proportions, unless stated otherwise. Groups compared using one-way analysis of variance with Tukey test for multiple comparisons or Student’s $t$ test for parametric data, Mann-Whitney U test for non-parametric data, and Chi-squared test or Fisher’s exact test for proportions. Significant differences across or between groups denoted *(p < 0.05) with trends towards significance denoted *(p < 0.1). Significant differences compared to healthy control group denoted *(p < 0.05) or ***(p < 0.01).
Table 2: Physiological, computed tomography and magnetic resonance data across participant groups

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 17)</th>
<th>Asthma S_{acin} normal (n = 20)</th>
<th>Asthma S_{acin} high (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; (% pred.)****</td>
<td>97.7 (3.4)</td>
<td>83.9 (3.8)</td>
<td>65.4 (4.8) ^####, ¥¥¥</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; z score****</td>
<td>-0.18 (0.23)</td>
<td>-1.16 (0.26) ^#</td>
<td>-2.28 (0.29) ^####, ¥¥</td>
</tr>
<tr>
<td>FVC (% pred.)**</td>
<td>107.7 (3.7)</td>
<td>94.4 (2.9) ^#</td>
<td>91.6 (3.3) ^#</td>
</tr>
<tr>
<td>FVC z score**</td>
<td>0.53 (0.27)</td>
<td>-0.42 (0.21) ^#</td>
<td>-0.66 (0.26) ^#</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC (%)****</td>
<td>72.1 (1.7)</td>
<td>70.6 (2.5)</td>
<td>55.6 (3.3) ^####, ¥¥¥¥</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC z score***</td>
<td>-1.03 (0.23)</td>
<td>-1.23 (0.30)</td>
<td>-2.73 (0.31) ^####, ¥¥</td>
</tr>
<tr>
<td>FRC (L)**</td>
<td>3.67 (0.26)</td>
<td>3.08 (0.23)</td>
<td>4.28 (0.26) **</td>
</tr>
<tr>
<td>FRC (% pred.)*</td>
<td>114.4 (6.2)</td>
<td>103.7 (6.7)</td>
<td>131.5 (6.2) **</td>
</tr>
<tr>
<td>TLC (L)</td>
<td>6.92 (0.48)</td>
<td>5.70 (0.34)</td>
<td>6.77 (0.39)</td>
</tr>
<tr>
<td>TLC (% pred.)</td>
<td>109.8 (3.7)</td>
<td>103.3 (3.8)</td>
<td>109.5 (3.4)</td>
</tr>
<tr>
<td>RV/TLC (%)****</td>
<td>31.9 (2.2)</td>
<td>38.2 (1.9)</td>
<td>48.3 (2.3) ^####, ¥¥</td>
</tr>
<tr>
<td>RV/TLC (% pred.)****</td>
<td>88.3 (3.6)</td>
<td>104.8 (4.2)</td>
<td>125.6 (6.0) ^####, ¥¥¥</td>
</tr>
<tr>
<td>VA/TLC (%)****</td>
<td>88.2 (1.8)</td>
<td>82.0 (1.9)</td>
<td>74.3 (1.9) ^####, ¥¥</td>
</tr>
<tr>
<td>KCO (mmol•min&lt;sup&gt;-1&lt;/sup&gt;•kPa&lt;sup&gt;-1&lt;/sup&gt;•L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.55 (0.06)</td>
<td>1.66 (0.06)</td>
<td>1.58 (0.07)</td>
</tr>
<tr>
<td>LCI****</td>
<td>7.34 (0.26)</td>
<td>7.43 (0.25)</td>
<td>9.59 (0.31) ^####, ¥¥¥</td>
</tr>
<tr>
<td>S_{cond} (L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.029 (0.004)</td>
<td>0.054 (0.015)</td>
<td>0.068 (0.012)</td>
</tr>
<tr>
<td>S_{acin} (L&lt;sup&gt;-1&lt;/sup&gt;)****</td>
<td>0.120 (0.012)</td>
<td>0.115 (0.011)</td>
<td>0.319 (0.026) ^####, ¥¥¥¥</td>
</tr>
<tr>
<td>MLD E/I</td>
<td>-</td>
<td>0.83 (0.02)</td>
<td>0.89 (0.01)</td>
</tr>
<tr>
<td>P&lt;sub&gt;15&lt;/sub&gt; (HU)</td>
<td>-</td>
<td>-941 (5)</td>
<td>-950 (5)</td>
</tr>
<tr>
<td>ADC 13ms (cm&lt;sup&gt;2&lt;/sup&gt;•s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.132 (0.006)</td>
<td>0.121 (0.005)</td>
<td>0.137 (0.005)</td>
</tr>
<tr>
<td>ADC 1s (cm&lt;sup&gt;2&lt;/sup&gt;•s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.017 (0.001)</td>
<td>0.019 (0.001)</td>
<td>0.024 (0.002)</td>
</tr>
</tbody>
</table>
FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; FRC = functional residual capacity from body plethysmography; TLC = total lung capacity; RV = residual volume; VA = alveolar volume from single breath helium dilution; KCO = carbon monoxide transfer coefficient; LCI = lung clearance index; MLD E/I = mean lung density expiratory / inspiratory ratio; P₁₅ = Fifteenth lower percentile of inspiratory lung attenuation curve; ADC = apparent diffusion coefficient.

Data expressed as mean (standard error). Groups compared using one-way analysis of variance with Tukey test for multiple comparisons or Student’s t test. Significant differences across groups denoted *(p < 0.05), **(p < 0.01), ****(p < 0.001) or *****(p < 0.0001). Significant differences compared to healthy control group denoted #(p < 0.05), ##(p < 0.01), ###*(p < 0.001) or ####*(p < 0.0001). Significant differences between asthma S₈₀-low and S₈₀-high groups denoted ♀(p < 0.05), ♂♀(p < 0.01), ♂♂♂*(p < 0.001) or ♂♂♂♂*(p < 0.0001).
**Figure legends**

**Figure 1: Schematic diagram of length scales probed by helium apparent diffusion coefficients**
Dotted line represents maximal anatomical sensitivity of SF$_6$ phase III slopes [41].

**Figure 2: Quantitative computed tomography densitometry between asthma groups**
Panels A and B display the quantitative assessment of expiratory air trapping in patients with low and high levels of air trapping, respectively. Coloured spheres represent areas of lung > 1 ml in volume with an attenuation on expiratory scans of < -856 Hounsfield units. Panels C and D display the ratio of mean lung density on expiration to inspiration (MLD E/I), and the fifteenth lower percentile of the inspiratory lung attenuation curve ($P_{15}$), respectively, in patients with asthma. Error bars indicate means ± standard errors of the mean.

**Figure 3: Apparent diffusion coefficients (ADC) across groups**
ADC at 13ms (Panel A) and 1s (Panel B) are shown across healthy and asthma groups. Error bars indicate means ± standard errors of the mean.

**Figure 4: Correlations between $^3$He-MR, CT and physiological variables in patients with asthma**
Correlations are shown between apparent diffusion coefficients and $S_{acin}$ (Panels A and B), functional residual capacity percent predicted (Panels C and D), and the ratio of mean lung density on expiration to inspiration (Panels E and F). Best-fit linear regression lines and Pearson correlation coefficients are shown.
Figure 5 Change in apparent diffusion coefficient (%) against change in volume of gas in the lungs (%) in healthy subjects and patients with asthma

Correlations are shown between percentage change in ADC and percentage change in volume of gas in the lungs in three healthy subjects (Panel A) and three patients with asthma (Panel B). The three participants in each case are denoted with different symbols. \( V \) = volume of gas in lungs; \( V_{FRC} \) = volume of gas in lungs at functional residual capacity; \( ADC_V \) = ADC at lung volume \( V \); \( ADC_{FRC} \) = ADC at functional residual capacity (extrapolated).
SF₆ maximal phase III slope anatomical sensitivity
Figure No.2 - Unmarked
Click here to download high resolution image