HIGHLIGHTED TOPIC | Imaging Lung Physiology

Airway closure on imaging relates to airway hyperresponsiveness and peripheral airway disease in asthma

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1Woolcock Institute of Medical Research, Glebe, New South Wales, Australia; 2The University of Sydney, Sydney, New South Wales, Australia; 3Cooperative Research Centre for Asthma and Airways, Glebe, New South Wales, Australia; 4Department of Respiratory Medicine, Royal North Shore Hospital, St. Leonards, New South Wales, Australia; 5Department of Nuclear Medicine, Royal North Shore Hospital, St. Leonards, New South Wales, Australia; and 6Department of Respiratory Medicine, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia

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Farrow CE, Salome CM, Harris BE, Bailey DL, Bailey E, Berend N, Young IH, King GG. Airway closure on imaging relates to airway hyperresponsiveness and peripheral airway disease in asthma. J Appl Physiol 113: 958–966, 2012. First published July 26, 2012; doi:10.1152/japplphysiol.01618.2011.—The regional pattern and extent of airway closure measured by three-dimensional ventilation imaging may relate to airway hyperresponsiveness (AHR) and peripheral airways disease in asthmatic subjects. We hypothesized that asthmatic airways are predisposed to closure during bronchoconstriction in the presence of ventilation heterogeneity and AHR. Fourteen asthmatic subjects (6 women) underwent combined ventilation single photon emission computed tomography/computed tomography scans before and after methacholine challenge. Regional airway closure was determined by complete loss of ventilation following methacholine challenge. Peripheral airway disease was measured by multiple-breath nitrogen washout from which $S_{cond}$ (index of peripheral conductive airway abnormality) was derived. Relationships between airway closure and lung function were examined by multiple-linear regression. Forced expiratory volume in 1 s was 87.5 ± 15.8% predicted, and seven subjects had AHR. Methacholine challenge decreased forced expiratory volume in 1 s by 23 ± 5% and increased nonventilated volume from 16 ± 4 to 29 ± 13% of computed tomography lung volume. The increase in airway closure measured by nonventilated volume correlated independently with both $S_{cond}$ (partial $R^2 = 0.22$) and with AHR (partial $R^2 = 0.38$). The extent of airway closure induced by methacholine inhalation in asthmatic subjects is greater with increasing peripheral airways disease, as measured by ventilation heterogeneity, and with worse AHR.

asthma; airway closure; airway hyperresponsiveness; single photon emission computed tomography; ventilation heterogeneity

Asthma is characterized by intermittent airway narrowing and airway closure, which cause typical symptoms of wheeze, breathlessness, and chest tightness. Airway closure occurs during bronchial challenge [measured by a decrease in forced vital capacity (FVC) (5, 9, 34) and an increase in closing capacity (CC) (43)] and is associated with more severe asthma in terms of greater risk of exacerbations (9, 14, 34). Airway hyperresponsiveness (AHR) in asthma is also associated with more severe and poorly controlled disease, greater risk of hospitalization (32), and lung function decline (35). AHR is characterized by loss of the maximal response plateau, which implies that asthmatic airways may narrow to complete closure (26). Despite important associations between excessive airway closure and poor clinical outcomes, the mechanisms behind closure are poorly understood, and the relationship between airway closure and AHR is unclear.

Airway closure, measured by decrease in FVC during bronchoconstriction, is associated with AHR independently of the extent of airway narrowing (5). However, spirometry is a global measurement and gives no information on the regional effects of closure. Indeed, a previous imaging study showed that, at baseline, the difference between asthmatic subjects and healthy controls is not in the volume of absent ventilation, but in its patchy distribution and the loss of its relationship with age (22). Furthermore, there are no published imaging studies on the association between either the magnitude or distribution of ventilation loss during bronchoconstriction and AHR.

Airway closure causes ventilation defects that are measurable in three-dimensional ventilation images, such as single photon emission computed tomography (SPECT), positron emission tomography, high-resolution computed tomography (CT), and magnetic resonance imaging (MRI) with hyperpolarized He-3 (13, 22, 33). The ventilation defects that are apparent on ventilation imaging, and could be caused by airway closure and/or severe regional narrowing, are clearly identifiable regions or clusters at baseline, with new regions developing with bronchoconstriction in asthmatic subjects. Recent results from computational modeling of asthmatic lungs suggest that the basis of regional airway closure is widespread disease of small airways (38). Ventilation distribution in asthmatic subjects is characteristically more uneven and heterogeneous (4), which, in turn, causes airways to be unstable during bronchoconstriction. This results in patchy narrowing (20) and widespread airway closure (37). However, these theories have not been confirmed by experimental data to determine if there is any association between markers of peripheral airway dysfunction and the development of widespread airway closure during bronchoconstriction.

We hypothesize that abnormal peripheral airway function at baseline, which increases ventilation heterogeneity, is associated with more extensive airway closure during bronchocon-
striction, leading to greater loss of ventilation. Furthermore, we hypothesize that the loss of ventilation during bronchoconstriction is increased in subjects with more severe AHR. The aim of the present study was to determine whether the extent of ventilation loss measured by ventilation single photon emission computed tomography (VSPECT)/CT during bronchoconstriction [standardized to 20% fall in forced expiratory volume in 1 s (FEV₁)] was related to the severity of AHR to methacholine and to the severity of peripheral airway disease, measured by multiple-breath nitrogen washout (MBNW), in asthmatic subjects. Some of the results of this work have been previously reported in abstract form (42).

**METHODS**

**Subjects.** Asthmatic subjects were recruited from the Department of Respiratory Medicine, Royal North Shore Hospital, the Woolcock Institute of Medical Research, and by local advertising. Asthma was defined as a respiratory physician diagnosis and a history of asthma symptoms and medication use within the last 6 mo. Exclusion criteria were current smoking and any history of smoking ≥10 pack-yr; FEV₁ < 60% predicted or 1.4 liters before bronchial challenge testing; and respiratory tract infection within the last 4 wk. All subjects provided written, informed consent, and the study was approved by the local Human Research Ethics Committee [protocol no. 0512–232M (SP)].

**Study design.** The study design is depicted in Fig. 1. Subjects attended for two visits, 2–7 days apart, and were instructed to withhold short-acting bronchodilators for at least 8 h and long-acting bronchodilators for at least 24 h before each visit. At the initial visit, subjects completed the Asthma Control Questionnaire (ACQ) (16), underwent baseline lung function, including a MBNW test, followed by a VSPECT/CT scan, and then completed a methacholine challenge test. At the second visit, an abbreviated methacholine challenge test was administered. The second VSPECT scan was immediately acquired after the FEV₁ decreased by 20% of the value recorded at the baseline scan. If baseline FEV₁ at the second visit differed by >10% compared with the baseline VSPECT/CT study, the subject did not proceed to scanning. Atopy was determined by skin prick testing.

**Lung function testing.** Spirometry and lung volumes were measured in accordance with European Respiratory Society/American Thoracic Society guidelines (29) using a Vmax 22 Autobox (Sensormedics, Yorba Linda, CA) and Medgraphics CPFS/D USB spirometer (Medgraphics, St. Paul, MN). MBNW was performed as previously described (8) from which lung clearance index (LCI), S.cond and S.acin (measures of ventilation heterogeneity in peripheral conducting and diffusion dependent airways, respectively) were derived (8, 39).

**MBNW test and analysis technique.** Briefly, after establishment of stable tidal breathing, subjects inhaled 1- to 1.3-liter tidal breaths at a rate of 8–12 breaths/min until expired nitrogen concentration was <2%. The phase III slopes of each washout breath were normalized by dividing by the mean expired nitrogen concentration. The expired volumes were normalized for lung size by dividing the cumulative expired volume, by the functional residual capacity (FRC) derived from the MBNW. FRC was determined by measurement of the total cumulative expired nitrogen at the end of the washout. Thus one lung turnover represents a cumulative expired volume equal to FRC. The normalized phase III slopes were then plotted against lung turnover. S.cond was determined by the slope of the linear regression between turnovers 1.5 and 6. S.acin was determined as the phase III slope of the first breath, minus the component attributed to conductive heterogeneity, based on the S.cond value (lung turnover value of the first breath × S.cond) (8). The LCI was determined as the number of lung turnovers at which mean expired nitrogen concentration reaches 4% of the baseline value and is a global index. S.cond is an index of ventilation heterogeneity in convection-dependent airways, while S.acin is an index in diffusion-convection dependent airways (8, 39).

**Methacholine challenge protocol.** Methacholine challenge was performed with cumulative doubling doses administered via a Kokos dosimeter (PDS Instrumentation, Louisville, KY), ranging from 0.06 to 200 μmol, with spirometry being measured 60 s after methacholine administration. At the second visit, an abbreviated challenge was performed, starting with the penultimate dose of the initial challenge, to achieve a 20% decrease in FEV₁. Pulse oximetry was monitored during methacholine challenge testing (BCI Fingerprint Pulse Oximeter, Waukesha, WI).

**AHR** was defined as a provocative dose that causes 20% decline in FEV₁ of ≤4 μmol. The severity of AHR was determined by the dose response ratio (%fall in FEV₁/final dose + 3), which is a continuous measure that allows inclusion of data in which <20% decrease in FEV₁ occurred. The constant “3” is added to avoid negative values in completely unresponsive subjects (30), which then allows log transformation for statistical analysis.

**Lung imaging.** VSPECT/CT was performed as previously described using Technegas as the ventilation agent (Cyclomedica Australia, Sydney, Australia) (11, 21). Technegas is a 100-nm radiola- beled carbon particle whose inhaled deposition has a similar distribution to a gas, but remains unchanged for 20 min or more (1, 23). This allows inhalation in the upright, seated posture with subsequent supine scanning. One-liter volumes of Technegas were slowly inhaled from FRC (~30–60 l/min), requiring two to four inhalations to achieve radioactivity of ~1.5 kilocounts/s required for imaging. The bag-in-box device allowed delivery of a 1-liter volume from a set prespiratory lung volume (in this case FRC), with respiratory flow rates being displayed in real time to ensure the subject did not go over the 1-liter inhalation (Fig. 2). VSPECT images were then acquired, immediately followed by a low-dose CT scan. Subjects remained stationary on the same gantry, since the two scanners were juxtaposed. VSPECT and CT were both acquired during resting tidal breathing. Total dose for the entire study was <4.9 mSv per subject.

The SPECT/CT machine was purpose-built, incorporating a single-slice, low-energy, high-resolution collimator scanner (Picker PQ5000 CT) and dual-detector variable-angle hybrid SPECT/CT system (Philips SKYLight) (12). All SPECT studies were acquired using a 128 × 128 matrix, at 15 s per stop with 3° steps over 360°. Low-dose CT was performed without contrast, with a low-dose protocol (30 mA, 120 kVp, pitch 1.5, slice thickness 4 mm).

**Image reconstruction and registration.** The ventilation study was reconstructed using ordered subset expectation maximization, with 128 slices and a Butterworth filter with a cut-off 0.8 and order 9. CT images are reconstructed using a 512 × 512 matrix with a Siemens

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**Study Design**

**Visit 1**  
- Baseline  
  - Questionnaire  
  - Respiratory Function Tests  
  - MBNW  
  - VSPECT/CT  
  - Methacholine Challenge

**Visit 2**  
- Methacholine Challenge  
  - Spirometry  
  - Modified Methacholine Challenge #  
  - VSPECT

*Fig. 1. Study design. The study was conducted over 2 visits that were a minimum of 48 h apart. The modified methacholine challenge was an abbreviated challenge, starting with the penultimate dose of the initial challenge, to achieve a 20% decrease in forced expiratory volume in 1 s (FEV₁) from baseline. MBNW, multiple-breath nitrogen washout; VSPECT/CT, ventilation single photon emission computed tomography.*
algorithm and transferred to a HERMES (Nuclear Diagnostics, Stockholm, Sweden) workstation in DICOM format. Registration of the SPECT ventilation study to CT scan was performed using the known, fixed spatial relationship between the SPECT and CT scanners. Additional three-dimensional registration was further performed using a HERMES Multimodality software package (Nuclear Diagnostics), which used a mutual information algorithm (12).

Image analysis. Airway closure was determined from the registered VSPECT and CT scans, as shown in Fig. 3A. A CT mask of less than 50% Hounsfield units was used to define the lung outline, which was then applied to the VSPECT image. The CT lung outline was the FRC measured from CT image data, as the total number of voxels × voxel volume. Any voxels in the VSPECT image dataset lying outside the lung outlines were set to zero and thus removed from further analysis. This eliminated localized spots of Technegas activity that lay outside the lung, such as the pharynx, esophagus, and stomach, which is a common occurrence in clinical VSPECT imaging.

Quantification of nuclear ventilation scans requires removal of noise due to photon scatter. This is achieved using a “threshold”, which is a single value derived from the distribution of Technegas activity. A threshold value is calculated individually from each scan, allowing normalization across all VSPECT scans. Deposition in locations of turbulence (typically in very proximal airways) causes “hot spots”, i.e., localized areas of high activity, which is another common artifact in nuclear medicine ventilation imaging. Neither scatter nor hot spots accurately represent ventilation and are a common occurrence in clinical VSPECT imaging.

The image analysis algorithm must, therefore, adapt to the wide variation in Technegas distributions for accurate volume measurements. Traditional threshold methods are based on the hot spot (20), which are inadequate for very heterogeneous ventilation that occurs in asthma (3).

Therefore, an adaptive threshold was used to calculate ventilated lung volume and eliminate noise due to scatter in the VSPECT image. This was done using the following steps on the VSPECT data set after applying the CT mask (see Fig. 3B): 1) voxels with activity values between 5 and 80% of maximum were identified; 2) the mean ventilation value from this data set was calculated; 3) 50% of this mean value was used as the threshold; and 4) anything less than this value in the original CT-masked data set was considered to be nonventilated lung and set to zero. In using this method, extreme values (scatter and hotspots) had negligible effect on the threshold value, so that it was then based on the vast majority of ventilated lung.

The threshold method was verified by comparing baseline ventilated volumes calculated from this method against FRCs derived from MBNW. Threshold values were typically 15–30% of peak values, depending on the skewness of the distribution of ventilation values. Lung volume was defined from the CT, while ventilated volume was defined from VSPECT. Airway closure was calculated as the nonventilated volume (NVV) on VSPECT (a measurement of airway closure and/or severe regional narrowing) and reported as a proportion of total lung volume determined from the CT (Fig. 4).

The topography of airway closure was defined in relation to its craniocaudal distribution by dividing the lung into thirds by axial length based on CT slice. NVV in each lung third was described as a percentage of the CT volume of that third to account for differences in regional volume. The ratio of closure (NVV) in the middle to lower thirds was used as a measure of distribution. A ratio > 1 indicates greater closure in the middle third.

Statistical analyses. Comparisons between closure at baseline and postmethacholine were examined using paired t-tests, significance P < 0.05. Data are presented as means ± SD, unless otherwise stated. The relationship between the ratio of middle to lower third closure and AHR was examined using the Spearman rank correlation. The relationships between the extent of airway closure and lung function parameters (spirometry, ventilation heterogeneity, and AHR), were examined using linear regression analyses. For an expected Pearson correlation coefficient of 0.65, we required 15 subjects for 80% power at the 0.05 level of significance (8). Data were analyzed using Analyse-It for Excel (Analyse-It Software, Leeds, UK).

RESULTS

Seventeen asthmatic subjects were recruited (age range 19–67 yr), but three subjects were subsequently excluded at the second visit as their baseline FEV1 differed from the initial visit by >10%. Characteristic for the remaining 14 subjects are shown in Table 1. There was a wide range of asthma severity, as determined by the wide range of AHR and spirometry values. Seven of fourteen subjects had AHR. At the second...
visit, FEV$_1$ was $88 \pm 14\%$ predicted, which was not significantly different from the first visit ($P \geq 0.05$). Measures of ventilation heterogeneity ($S_{\text{cond}}, S_{\text{acins}},$ and LCI) obtained from MBNW were similar to previously published data in asthmatic subjects (8). Nine of fourteen subjects were taking regular inhaled corticosteroids, and eight of fourteen subjects were also taking long acting β-agonist. Asthma was well to moderately controlled in the majority with ACQ scores ranging from 0.3 to 1.7.

The volume of the entire lung calculated from CT acquired at FRC was 2.96 ± 0.84 liters and correlated with plethysmographic FRC of 3.13 ± 0.94 liters ($r = 0.84, P = 0.0002$). Figure 5A shows the comparison using Bland and Altman plots, which indicate that there is no systematic difference in measurement between the two methods. The mean ventilated volume measured from VSPECT was 2.46 ± 0.61 liters and correlated with mean nitrogen washout FRC of 2.47 ± 0.63 liters ($r = 0.76, P = 0.0015$), but not with plethysmographic
FRC. Both VSPECT volume and nitrogen washout FRC are dependent on airways that are open to the mouth during tidal breathing. Figure 5B shows that there were no systematic differences in measurement between the two methods. The baseline NVV representing airway closure was 16 ± 4% of the total volume. At baseline, airway closure did not correlate with FVC (%predicted), ventilation heterogeneity, or AHR.

Methacholine challenge caused significant reduction in spirometric function and an increase in airway closure on imaging, as seen in fused images (Fig. 4). The mean methacholine dose given at the second visit was 6.5 ± 9 μmol. The mean decrease in FEV₁ was 22.6 ± 5% of baseline (range 17–35%, P < 0.0001) and in FVC was 15 ± 6% (range 7–28%, P < 0.0001). The FEV₁-to-FVC ratio decreased from 73.2 ± 8.9 to 66.2 ± 8.6% (P = 0.001), and oxygen saturation, measured by pulse oximetry, was unchanged (P ≥ 0.05). After methacholine challenge, mean ventilated volume from VSPECT was 2.07 ± 0.53 liters, and airway closure increased from 16 ± 4% at baseline to 29 ± 13% of total lung volume (P = 0.002).

The change in airway closure measured by NVV, induced by methacholine challenge, correlated with AHR (r = 0.56, P = 0.04) (Fig. 6A) and with the three indexes of ventilation heterogeneity at baseline (Scond: r = 0.58, P = 0.028, Fig. 6B; Sacin: r = 0.60, P = 0.025; LCI: r = 0.69, P = 0.007). However, this change in NVV did not correlate with changes in FEV₁ or FVC (r = 0.05, P = 0.85 and r = 0.10, P = 0.74, respectively). In a multivariate analysis, the change in NVV induced by methacholine correlated independently with AHR and with Scond (total R² = 0.60, P = 0.007). The partial R² values were 0.22 for Scond and 0.38 for AHR.

Airway closure measured by NVV at baseline was greater in the lower compared with the middle third of the lung (23 ± 11 and 9 ± 2.6% of regional lung volume, P < 0.0001) (Fig. 7). The extent of NVV at baseline in the upper third (23 ± 6.2%) was greater than in the middle third (P < 0.0001), but equal to that in the lower third. After methacholine challenge, NVV in the lower third (48 ± 23.6%) was greater than NVV in both the middle (21 ± 13.5%, P = 0.0003) and upper thirds (26 ± 18%, P = 0.02). Therefore a gravitational gradient occurred after induced airway closure, with no increase in NVV in the upper third (3 ± 18%), but increased NVV in the middle (12 ± 14%) and the lower thirds (25 ± 22%). The ratio of NVV in the middle to lower thirds, either at baseline or after methacholine challenge, did not correlate with age, AHR, spirometry, indexes of ventilation heterogeneity measured by MBNW, body mass index, or asthma control score.

Table 1. Subject characteristics from visit 1

<table>
<thead>
<tr>
<th>Sex (M/F)</th>
<th>8/6</th>
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</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>40.9 ± 18.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.5 ± 4.1</td>
</tr>
<tr>
<td>FEV₁, %predicted</td>
<td>87.6 ± 15.8</td>
</tr>
<tr>
<td>FVC, %predicted</td>
<td>99.4 ± 10.5</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>73.3 ± 8.9</td>
</tr>
<tr>
<td>DRR mean (range), %decrease FEV₁/μmol</td>
<td>0.068 ± 0.04</td>
</tr>
<tr>
<td>Scond, liter</td>
<td>0.193 ± 0.06</td>
</tr>
<tr>
<td>LCI (lung turnovers), no.</td>
<td>10.3 ± 2.25</td>
</tr>
<tr>
<td>Asthma control score</td>
<td>0.78 ± 0.53</td>
</tr>
</tbody>
</table>

Values are means ± SD. M, male; F, female; Y, yes; N, no; BMI, body mass index; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; DRR, dose response ratio; Scond and Sacin, measures of ventilation heterogeneity in peripheral conducting and diffusion dependent airways, respectively; LCI, lung clearance index.
Although AHR and ventilation heterogeneity at baseline both correlated with the change in airway closure from VSPECT after challenge, they were not correlated with each other (AHR vs. \( S_{\text{cond}} \): \( r = 0.13, P = 0.66 \); AHR vs. \( S_{\text{acin}} \): \( r = 0.29, P = 0.32 \); AHR vs. LCI: \( r = 0.38, P = 0.18 \)). AHR was also unrelated to residual volume/total lung capacity, FRC/total lung capacity, and FEV\(_1\)/FVC. The ACQ score did not correlate with dose-response ratio, VSPECT airway closure at baseline, or the change induced by methacholine challenge.

**DISCUSSION**

In this study of asthmatic subjects, the extent of airway closure induced by methacholine challenge was related to the severity of ventilation heterogeneity at baseline. Furthermore, we found that subjects with more severe AHR had the greatest NVV, despite a similar level of bronchoconstriction to those subjects with mild or no AHR. Additionally, there was greater NVV in the lower third of the lung, but its distribution, in relation to middle third or lower third predominance, was not associated with AHR, ventilation heterogeneity, or asthma severity. The significant relationship demonstrated between induced NVV, due to severe airway narrowing and closure measured by VSPECT/CT, and AHR extends the observations of Chapman et al. (5), as well as the association between airway closure and severe asthma (9, 14). In addition, the correlation between NVV and \( S_{\text{cond}} \) suggests that peripheral airway dysfunction may predispose to severe airway narrowing and closure during bronchoconstriction.
The correlation between the increase in NVV and heterogeneity at baseline supports a previously published model that suggests regionally clustered airway closure results from ventilation heterogeneity (38). In this model, ASM activation leads to widespread airway narrowing, which, in a heterogeneous airway tree, causes large regions of airways to become unstable, leading to airway closure. It has been suggested that this regional instability is secondary to the interaction of forces between airways in close proximity. Consequently, narrowing of airways is highly interdependent. Further modeling suggests that closure is dependent on widespread heterogeneous peripheral airway narrowing with severe narrowing or closure in more proximal airways (37).

The development of nonventilated regions due to severe airway narrowing and closure during bronchoconstriction (Fig. 4) has been observed in previous ventilation imaging studies in which ventilation was measured in a variety of ways (7, 31, 33, 36). In the present study, the concurrent low-dose CT scan was used to define the anatomical outline of the lung, and VSPECT was used to define ventilation, allowing objective measurement of NVV in relation to CT lung volume. This was an improvement of a previously validated method, where VSPECT closure correlated closely with CC in healthy subjects (21). Although the low-dose CT did not allow further density measures for heterogeneity, FRC measured by CT and by plethysmography were similar. Since FRC was likely reduced in supine posture during CT (27), it was surprising that CT-measured FRC was not less than FRC measured by body plethysmography. This may be due to overestimation of CT lung volume due to the inclusion of blood vessels and to blurring of the lung borders due to breathing artifact. VSPECT measures only lung regions that communicate with the mouth, and it is reassuring that it is similar to MBNW FRC. Nevertheless, some errors in registration of the VSPECT and CT images may have occurred, which would have affected the measurement of NVV. The misregistrations would have unpredictable effects on the measurements of NVV, but are unlikely to systematically cause a spurious relationship between NVV and S$_{cond}$ and AHR.

The contribution of imaging to understanding airway closure and AHR is its topographical localization. In the present study, subjects who have worse AHR had a larger volume of lung that closed at similar falls in FEV$_1$ of ~20%. Baseline closure, however, did not correlate with AHR. We speculate that the lung regions that closed may be responsible for driving AHR, whereas airways already closed at baseline may already be near their maximal response and may not be capable of any further augmentation with bronchoconstriction. This topographical construct is consistent with the known dysfunction of peripheral airways in asthma (5, 8, 15, 17, 18, 41) and its association with AHR (5, 8). Closure occurs both peripherally and centrally (8, 10) and could be due to worse inflammation, edema, mucous production, surfactant abnormalities, airway wall thickening, and changes in wall compliance (19, 40). By subjective assessment, airway closure occurs at the same location on different days (6). Confirmation by objective analyses and reduction by anti-inflammatory treatment, in parallel with improvements in AHR, would be needed to determine whether closure was due to localized rather than diffuse or transient phenomena, such as mucous or surfactant abnormalities. This would have implications for inhaled treatment with respect to targeting aerosol treatment to specific locations.

Airway closure measured by VSPECT and by FVC did not correlate in the present study and is likely to represent different aspects of function. Similarly, closure measured by VSPECT correlates with single-breath CC in normal subjects (21), but does not correlate in asthmatic subjects (22). In a hyperpolarized He-3 MRI study, methacholine-induced closure measured subjectively as the average number of defects per MRI slice (6), correlated with spirometry when pre- and postmethacholine data were pooled. There were similar findings in the present study between pooled data of FVC %predicted and airway closure ($r = -0.47, P = 0.01$), indicating that, as expected, bronchoconstriction decreased FVC and increased the extent of airway closure on VSPECT. However, there were no such relationships in either premethacholine or postmethacholine data analyzed separately, nor in the changes. Thus pooling data of two different physiological states may be of dubious significance. The absence of relationship between FVC and VSPECT measured closure may also be because Technegas was inhaled as a 1-liter bolus from FRC and because of volume history. The VSPECT imaging, therefore, reflects the extent of closure at FRC on inspiration (so that a comparison with MBNW can be made), whereas changes in FVC are due to airways closing during forced expiration, usually below FRC. Thus the differences in physiological conditions in which spirometry and VSPECT imaging occur make any correlations between changes in FVC and imaging unlikely. The spatial resolution of the SPECT images is ~15 mm$^2$ and could affect these relationships. We were only able to detect closure that affected lung regions of ~4–5 ml in volume. Smaller subresolution areas affected by closure were probably present and would contribute to the reduction in FVC. Finally, NVV may be due to closure and severe narrowing, and the effects of these two phenomena on NVV may differ to their effects on FVC, leading to a disassociation.

We hypothesized that the responsive airways that close occurred anywhere in lung, rather than being predominantly basal (21, 22), and that the distribution of closure (described in gravitational thirds) was related to AHR. Our hypothesis was
not supported, and we observed greater airway closure in the lower third of the lung. Therefore, although closure may be patchy in asthmatic subjects, it still follows a vertical distribution, suggesting that the gradient of elastic recoil operates in asthma as it does in normal subjects, which is in keeping with early distribution studies (3). Nevertheless, there could also be greater inflammation and remodeling in the lower third, similar to chronic obstructive pulmonary disease (2). The NVV in the upper third of the lung was greater than in the middle third. The difference was unlikely to be due to more airway closure in the upper third, but due to high lung recoil pressures, such that alveoli are closer to full expansion at FRC (28). Thus a 1-liter breath would minimally distend alveoli, resulting in very low specific ventilation and minimal Technegas deposition, perhaps below the lower limit of detection of our technique.

Technegas was inhaled in the upright posture, but VSPECT were acquired in the supine posture. Although the change in posture after Technegas inhalation may have affected the measured NVV, due to changes in regional lung inflation (27), this effect was likely to be small, as suggested by previous validation (21). If anything, differences in posture may have weakened the associations between closure and AHR and ventilation heterogeneity. Although Technegas was inhaled slowly, some nonventilated regions on VSPECT may be due to severe airway narrowing rather than true closure. The nitrogen washout phase 3 slopes, however, represent differences in regional time constants. Therefore, some of the relationship between S_\text{cond} (derived from the nitrogen washout phase 3) and airway closure by VSPECT may be related to severe regional airway narrowing, resulting in very long local time constants. The strong relationship between CC and SPECT airway closure, however (21), suggests that the relationship is driven predominantly by closure rather than narrowing. Four subjects did not have AHR, as defined by a provocative dose that causes 20% decline in FEV_1 < 4 μmol. Although it is possible they may not have asthma (24), their lack of AHR was likely due to continued inhaling corticosteroid treatment (25).

In summary, airway closure in asthmatic subjects induced by bronchoconstriction is related independently both to AHR and to ventilation heterogeneity. These data provide support for computational model predictions that suggest that airways tend to close rather than narrow when peripheral airway disease is worse, and that this closure is greater with more severe AHR. These findings have potential implications for the underlying mechanisms of AHR, in particular whether the location of induced closure is reproducible in asthma and if it is affected by anti-inflammatory treatment. Further studies should examine these questions and also compare asthmatic imaging data with nonasthmatic normal subjects.

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