The structural basis of airways hyperresponsiveness in asthma

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Brown, Robert H., David B. Pearse, George Pyrgos, Mark C. Liu, Alkis Togias, and Solbert Permutt. The structural basis of airways hyperresponsiveness in asthma. J Appl Physiol 101: 30–39, 2006. First published February 9, 2006; doi:10.1152/japplphysiol.01190.2005.—We hypothesized that structural airway remodeling contributes to airways hyperresponsiveness (AHR) in asthma. Small, medium, and large airways were analyzed by computed tomography in 21 asthmatic volunteers under baseline conditions (FEV1 = 64% predicted) and after maximal response to albuterol (FEV1 = 76% predicted). The difference in pulmonary function between baseline and albuterol was an estimate of AHR to the baseline smooth muscle tone (BSMT). BSMT caused an increase in residual volume (RV) that was threefold greater than the decrease in forced vital capacity (FVC) because of a simultaneous increase in total lung capacity (TLC). The decrease in FVC with BSMT was the major determinant of the baseline FEV1 (P < 0.0001). The increase in RV correlated inversely with the relaxed luminal diameter of the medium airways (P = 0.009) and directly with the wall thickness of the large airways (P = 0.001). The effect of BSMT on functional residual capacity (FRC) controlled the change in TLC relative to the change in RV. When the FRC increased with RV, TLC increased and FVC was preserved. When the relaxed large airways were critically narrowed, FRC and TLC did not increase and FVC fell. With critical large airways narrowing, the FRC was already elevated from dynamic hyperinflation before BSMT and did not increase further with BSMT. FEV1/FVC in the absence of BSMT correlated directly with large airway luminal diameter and inversely with the fall in FVC with BSMT. These findings suggest that dynamic hyperinflation caused by narrowed large airways is a major determinant of AHR in asthma.

ASTHMA IS CHARACTERIZED BY airways hyperresponsiveness (AHR), a manifestation of reversible airway obstruction from smooth muscle constriction. There is an increasing recognition that many patients with asthma also suffer from chronic airflow limitation that is resistant to conventional bronchodilating and anti-inflammatory therapies (40, 41). It has been suggested that the pathological changes in the airways responsible for chronic airflow obstruction may also influence AHR (36), but little is known about the relationship between airway structure and the chronic abnormal pulmonary function in asthma. We hypothesized that the structural changes of asthmatic airways that represent what many investigators in the field refer to as “remodeling” (23, 37) contribute significantly to the abnormalities of pulmonary function associated with asthma, including the phenomenon of AHR.

To test this hypothesis, we assessed the contribution of airway structural alterations in asthma to AHR, with the assumption that AHR can be estimated by the effect on pulmonary function of the amount of airways smooth muscle activation associated with the presence of baseline smooth muscle tone (BSMT). To accomplish this, we measured airway structure by high-resolution computed tomography (HRCT) and pulmonary function in 21 asthmatic volunteers before and after maximal bronchodilation with inhaled albuterol. We evaluated luminal diameters and wall thicknesses of airways for three airway sizes. We used the difference in pulmonary function before and after albuterol to quantify the magnitude of the airways response to BSMT and determined whether there was a relationship between the dimensions of the airways and the magnitude of the pre- to postalbuterol differences in pulmonary function.

We found that airway structure played a major role in the magnitude of AHR through different effects on airway closure [residual volume (RV)], on the one hand, and the magnitude of the increase in total lung capacity (TLC), on the other, with the summation of these effects determining the magnitude of the decrease in forced vital capacity (FVC). We propose that some of the major features of AHR in asthma and its absence in health are the result of the relationship between airways structure and the changes in TLC and RV that occur with increasing smooth muscle tone.

Glossary

- AHR: Airway hyperresponsiveness
- ALB: Albuterol
- BSMT: Baseline smooth muscle tone
- DiTLCalb (large): Large airway diameter measured at TLC after albuterol
- DiTLCalb (medium): Medium airway diameter measured at TLC after albuterol
- DiTLCalb (small): Small airway diameter measured at TLC after albuterol
- DiTLCbsmt (large): Large airway diameter measured at TLC with baseline tone
- DiTLCbsmt (medium): Medium airway diameter measured at TLC with baseline tone
- DiTLCbsmt (small): Small airway diameter measured at TLC with baseline tone
- FEV1: Forced expiratory volume in 1 s
- FRC: Functional residual capacity
- FRC_ratio: (FRCbsmt/FRCalb)/(RVbsmt/RValb)
- FVC: Forced vital capacity

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Pulmonary function measurements. Spirometric measurements were performed in triplicate with the subjects in a seated position using a portable Koko spirometer (PDS, Louisville, CO). Immediately after the spirometry measurements, lung volumes were measured in a body plethysmograph (MedGraphics, St. Paul, MN) with the subjects in a seated position. The volunteers were instructed to pant against the closed shutter at a frequency of 1–3 Hz. Most of the volunteers could not tolerate more than three sets of maneuvers in the box. The best quality set of measurements from a single maneuver was utilized. The volunteers underwent computed tomography (CT) scanning within 3–5 min of plethysmography.

We operationally defined the RV as the volume that remains in the lung at the end of a FVC. Therefore, the RV was considered to be the difference between the TLC and the FVC. There was no significant difference between the FVC and the slow vital capacity determined during the lung volume measurements, before or after the inhalation of albuterol.

Albuterol treatment. After recording baseline spirometric and plethysmographic values, each volunteer received nebulized albuterol (2.5 ml of 0.083%) over ~20 min, after which spirometric and thoracic gas volume measurements were repeated. The nebulized albuterol treatment was repeated until either the increase in FEV1 between treatments was <5% (plateau effect) or the heart rate increased above 140 beats/min. All volunteers reached a plateau before a heart rate of 140 beats/min was reached.

HRCT image acquisitions. All scans were performed using spiral CT (Somatom Plus 4, Siemens) with settings of 120 kVp, 170 mA, 2-mm slice thickness, rotation feed of 2 mm/s, and a reconstruction interval of 1 mm (total 61 scans per set) during a single breath hold for ~24 s at FRC and at TLC (see below) (4). Scanning began ~6 cm above the top of the dome of the diaphragm, at FRC, and moved caudally. A reference scan was acquired before each spiral CT scan set to ensure reproducible image location in the lung. The images were reconstructed as a 16-bit 512 × 512 matrix using a field of view of 200 mm. Images were reconstructed with the use of a high-spatial frequency (resolution) algorithm that enhanced edge detection, at a window level of ~450 Hounsfield units and a window width of 1,350 Hounsfield units. All airways visualized approximately perpendicular to the scan plane (long-to-short axis ratio <1.5:1) were measured. For

### Table 1. Demographic characteristics of all subjects

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Gender</th>
<th>Skin Test</th>
<th>PC20</th>
<th>FEV1, %Pred</th>
<th>FVC, %Pred</th>
<th>FEV1/FVC</th>
<th>Medications</th>
</tr>
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<tbody>
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<td>F</td>
<td>+</td>
<td>2.11</td>
<td>75</td>
<td>79</td>
<td>0.78</td>
<td>1, 2, 3, 6</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>M</td>
<td>+</td>
<td>1.83</td>
<td>80</td>
<td>105</td>
<td>0.62</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>F</td>
<td>+</td>
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<td>0.82</td>
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<tr>
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<td>+</td>
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<td>82</td>
<td>105</td>
<td>0.67</td>
<td>1, 2, 3, 6</td>
</tr>
<tr>
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<td>64</td>
<td>78</td>
<td>0.67</td>
<td>1, 2, 6</td>
</tr>
<tr>
<td>6</td>
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<td>F</td>
<td>+</td>
<td>0.91</td>
<td>78</td>
<td>75</td>
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<td>1, 3, 6</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>F</td>
<td>NP</td>
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<td>79</td>
<td>87</td>
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</tr>
<tr>
<td>8</td>
<td>25</td>
<td>F</td>
<td>NP</td>
<td>0.81</td>
<td>76</td>
<td>81</td>
<td>0.81</td>
<td>1, 6</td>
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<td>0.16</td>
<td>79</td>
<td>87</td>
<td>0.73</td>
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<td>+</td>
<td>0.81</td>
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<td>81</td>
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<td>M</td>
<td>+</td>
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<td>+</td>
<td>61.37</td>
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<td>44</td>
<td>M</td>
<td>+</td>
<td>0.30</td>
<td>60</td>
<td>60</td>
<td>0.40</td>
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<tr>
<td>18</td>
<td>34</td>
<td>F</td>
<td>+</td>
<td>0.65</td>
<td>73</td>
<td>85</td>
<td>0.54</td>
<td>1, 2, 3, 6</td>
</tr>
<tr>
<td>19</td>
<td>20</td>
<td>M</td>
<td>+</td>
<td>0.13</td>
<td>70</td>
<td>98</td>
<td>0.59</td>
<td>1, 6</td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>M</td>
<td>+</td>
<td>0.13</td>
<td>50</td>
<td>50</td>
<td>0.56</td>
<td>1, 3, 6</td>
</tr>
<tr>
<td>21</td>
<td>44</td>
<td>F</td>
<td>+</td>
<td>0.13</td>
<td>50</td>
<td>50</td>
<td>0.56</td>
<td>1, 3, 6</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td></td>
<td>39 ± 3</td>
<td>4.75 ± 3</td>
<td>67 ± 4</td>
<td>83 ± 4</td>
<td>0.66 ± 0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M, male; F, female; PC20, provocative concentration (mg/ml) of methacholine that causes a 20% fall in forced expiratory volume in 1 s (FEV1) from baseline; %Pred, percent predicted; FVC, forced vital capacity; NP, not performed; ☺, not challenged with methacholine. Skin test was for 12 common local aerallergens (Mixed Trees, Mixed Grasses, Ragweed, Alternaria, Cladosporium, D. farinae, D. pteronyssinus, Cat, Dog, B. germanica, B. orientalis, P. americana). Medications: 1, inhaled β-agonist bronchodilators; 2, inhaled steroids; 3, nasal steroids; 4, oral steroids; 5, theophylline; 6, antihistamines; 7, leukotriene receptor antagonists.

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repeated airway measurements in a given subject, within each experimental protocol, adjacent anatomic landmarks, such as airway or vascular branching points, were defined on the baseline HRCT images and again identified on the TLC scans and on the scans after the administration of albuterol (7).

Airway measurements with HRCT. Airway dimensions were derived from airway luminal area measurements. The airway luminal area measurement methodology has previously been described (8) and validated (1). Briefly, the HRCT images were transferred to a UNIX-based workstation and analyzed using the airway analysis module of the Volumetric Image and Display Analysis software package (Department of Radiology, Division of Physiologic Imaging, University of Iowa, Iowa City, IA). To measure airway luminal areas, the operator drew a rough isocontour estimate of the lumen of the airway. The software program automatically located a precise isocontour perimeter of the airway lumen by sending out rays in a spoke-wheel fashion to a predesignated pixel intensity level that defined the luminal edge of the airway wall. The length of the rays was set at 6 pixels. The software program used an algorithm for edge detection based on the “full-width-half-maximum” principle. The edge of the wall was defined by the points along the lines where the pixel intensity changed to one-half its maximum through the wall. All full and partial pixels (full pixel size equals 0.1537 mm² with our settings) within the adjusted isocontour were counted and represented the airway luminal area. The values for the airway wall diameter were obtained by calculating the diameter from the area measurement, assuming a circular structure, and were converted to millimeters by multiplying by the pixel dimension in millimeters.

To measure airway wall thickness (Wth), at least three lines were randomly drawn through the airway wall. The program automatically displayed a histogram of the pixel intensity along that line. The inflection points of increased intensity along the line that represents the inner and outer edges of the airway wall were selected, and the program then automatically measured the distance in pixels between the two points. The values for the Wth were converted to millimeters by multiplying by the pixel dimension in millimeters and averaged.

We measured between 20 and 41 airways in each volunteer. For nomenclature of the HRCT measurements of the airways, we divided the airways by their diameter at TLC at baseline (BSMT) into small (<5 mm in diameter), medium (5–10 mm in diameter), and large (>10 mm in diameter). We measured small and medium airways in all 21 volunteers, but we were able to measure a sufficient number of large airways in only 15 of the volunteers. We determined the diameter in millimeters after maximum relaxation with albuterol at TLC: DiTLCbsmt(small), DiTLCbsmt(medium), and DiTLCbsmt(large) for the small, medium, and large airways, respectively. We also determined the diameters at TLC under baseline conditions: DiTLCbsmt(small), DiTLCbsmt(medium), and DiTLCbsmt(large) for the small, medium, and large airways, respectively. Wth was measured at FRC before and after albuterol for each of the three airway sizes. Wall thickness was not significantly affected by albuterol (P > 0.05). Therefore, we only utilized the postalbuterol measurements designated WthFRCbsmt(small), WthFRCbsmt(medium), and WthFRCbsmt(large). We also measured the difference in the airway diameters at TLC between the albuterol and baseline conditions for the three airway sizes. We focused on a total of 12 airway measurements for each volunteer: three diameters at TLC under albuterol and baseline conditions for a total of six, three differences between the albuterol and baseline conditions, and three wall thicknesses at FRC with albuterol. We have no measurements of the wall thicknesses at TLC, because our initial focus was on the FRC measurement. It was not until the data were analyzed that the significance of the TLC measurements became apparent.

We consider the airway diameters after maximum relaxation with albuterol to be a function principally of the intrinsic structural characteristics of airways, because the effects of smooth muscle tone have been eliminated. We also had measurements of the diameters at FRC, but we inferred that the change in the diameter at TLC with changes in lung volume would be minimized due to the stiffness of the airways at TLC. Therefore, we considered the TLC measurements after albuterol to be the best reflection of intrinsic structure (22).

Protocols. HRCT scans were acquired at baseline FRC and TLC (i.e., with BSMT). On a separate day, after the plateau of the airway smooth muscle relaxing effect of nebulized albuterol treatment was obtained, HRCT scans were again acquired at FRC and TLC (relaxed airways).

Data analysis. Pulmonary function measurements in this study are expressed as either percent or fraction predicted values (http://www.hopkinsmedicine.org/pftlab/pfpred.htm). In a few instances, only absolute values are reported. For both lung function and structural measurements, the effect of BSMT was expressed as the percent change as determined by

\[
\% \text{ change} = \left[ \frac{(\text{BSMT value} - \text{ALB value})}{\text{ALB value}} \right] \times 100
\]

Comparisons between the mean values of pulmonary function and predicted values and between the percent predicted pulmonary function values before and after albuterol were made by two-tailed t-test. Least squares regression models were constructed to relate airway structure and function with the JMP software program (www.jmpdiscovery.com). Significance was accepted at a two-tailed level of P < 0.05.

RESULTS

Effect of BSMT on pulmonary function. There was a highly significant difference between each of the baseline pulmonary function variables of this study and their corresponding post-albuterol values (Fig. 1). Furthermore, all pulmonary function variables, except for the baseline TLC, baseline FRC, and albuterol RV, were significantly different from their predicted values. This was in keeping with the selection of volunteers with moderate to severe asthma.

With the assumption that the albuterol abolished the smooth muscle tone that was present under the baseline conditions, the BSMT can be viewed as having produced significant changes in all of the lung volume measurements (Fig. 1). The ranking of the changes in lung volumes produced by the BSMT indicated that the greatest change occurred in the RV, whereas the smallest change occurred in the FVC (Table 2). However, the percent change (%Δ) in FVC was the pulmonary function measurement that was the most closely correlated with both
%ΔFEV₁ (r = 0.86, P < 0.0001) (Fig. 2A) and the baseline FEV₁ fraction predicted (r = 0.77, P < 0.0001) (Fig. 2B).

Since large changes in both TLC and RV were less correlated with the FEV₁ than the small difference between them (i.e., the FVC), we can infer that the baseline FEV₁ must also have been a function of how much the TLC increased relative to the RV with the BSMT. The mean difference between BSMT and albuterol in the absolute RV of 0.907 liters was more than threefold greater than the 0.272-liter difference in the FVC. The small difference in FVC compared with the threefold greater difference in RV was the result of compensation by an increase in TLC of 0.634 liters induced by BSMT.

The change in FRC with BSMT played a dominant role in the control of the change in TLC relative to the change in RV. The strongest correlation between the change in the TLC and the change in any other pulmonary function measurement was with FRC (r = 0.93, P < 0.0001; Fig. 3A). The strongest correlation between the change in RV and the change in any other pulmonary function measurement was also with the change in FRC (r = 0.88, P < 0.0001; Fig. 3B). Thus the change in FRC with BSMT closely tracked the changes in RV (Fig. 3B) and TLC (Fig. 3A).

The FRC<sub>ratio</sub> and TLC<sub>ratio</sub>. Assuming that the effects of BSMT primarily impact the RV, we quantified the above relationships by formulating indexes that reflected the magnitude of the BSMT-related changes in FRC and TLC relative to the change in RV from BSMT that we defined as the FRC<sub>ratio</sub> and TLC<sub>ratio</sub>, respectively. The effect of the BSMT on the FRC was quantified by the ratio of the BSMT FRC to the albuterol FRC (FRC<sub>bsmt</sub>/FRC<sub>alb</sub>). The effect of the BSMT on the RV was quantified by the ratio of the BSMT RV to the albuterol RV, respectively.
RV (RV<sub>bsmt/RV<sub>alb</sub></sub>). The effect of the BSMT on the TLC was quantified by the ratio of the BSMT TLC to the albuterol TLC (TLC<sub>bsmt/TLC<sub>alb</sub></sub>). The ratio of FRC<sub>bsmt/FRC<sub>alb</sub></sub> to RV<sub>bsmt/RV<sub>alb</sub></sub> defines the FRC<sub>ratio</sub>. Thus,

\[
FRC_{\text{ratio}} = \frac{\text{FRC}_{\text{bsmt}}}{\text{FRC}_{\text{alb}}} / \frac{\text{RV}_{\text{bsmt}}}{\text{RV}_{\text{alb}}}
\]

Similarly,

\[
\text{TLC}_{\text{ratio}} = \frac{\text{TLC}_{\text{bsmt}}}{\text{TLC}_{\text{alb}}} / \frac{\text{RV}_{\text{bsmt}}}{\text{RV}_{\text{alb}}}
\]

These ratios are the same whether the absolute or fraction-predicted values of FRC, TLC, and RV are used. Our hypothesis was that the FRC<sub>ratio</sub> should be a determinant of the TLC<sub>ratio</sub>, and both should be determinants of the %ΔFVC. Figure 4 shows the correlation between FRC<sub>ratio</sub> and TLC<sub>ratio</sub> to be highly significant (r = 0.84, P < 0.0001). Moreover, the %ΔFVC significantly correlated with both the FRC<sub>ratio</sub> (r = 0.57, P = 0.007) (Table 3) and TLC<sub>ratio</sub> (r = 0.65, P = 0.001).

The logarithm of the concentration of methacholine that produced a 20% decrease in FEV<sub>1</sub> (log<sub>10</sub>PC<sub>20</sub>) in the 14 volunteers who were challenged was significantly correlated with the %ΔFVC that occurred from BSMT (r = 0.62, P = 0.018; Fig. 5), suggesting that the mechanism(s) that produced the BSMT acted in an analogous way to the inhaled methacholine on the FEV<sub>1</sub>. Furthermore, log<sub>10</sub>PC<sub>20</sub> was also significantly correlated with the FRC<sub>ratio</sub> (r = 0.66, P = 0.0108, Table 3). In addition to %ΔFVC, the FRC<sub>ratio</sub> was significantly correlated with other effects of the BSMT on pulmonary function: %ΔFEV<sub>1</sub> and %ΔRV (r = 0.50 and −0.74, respectively; P = 0.020 and 0.0001, respectively; Table 3).

Table 3. Pairwise correlations of FRC<sub>ratio</sub> with pulmonary function indexes of airway tone development

<table>
<thead>
<tr>
<th>Variable by Variable</th>
<th>Correlation</th>
<th>Count</th>
<th>Significant Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRC&lt;sub&gt;ratio&lt;/sub&gt;</td>
<td>log&lt;sub&gt;10&lt;/sub&gt;PC&lt;sub&gt;20&lt;/sub&gt;</td>
<td>0.66</td>
<td>14</td>
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<tr>
<td>FRC&lt;sub&gt;ratio&lt;/sub&gt;</td>
<td>%ΔFEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.50</td>
<td>21</td>
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<tr>
<td>FRC&lt;sub&gt;ratio&lt;/sub&gt;</td>
<td>%ΔFVC</td>
<td>0.57</td>
<td>21</td>
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<tr>
<td>FRC&lt;sub&gt;ratio&lt;/sub&gt;</td>
<td>%ΔRV</td>
<td>−0.74</td>
<td>21</td>
</tr>
</tbody>
</table>

See Glossary for definition of FRC<sub>ratio</sub>.

The conventional explanation for why the FVC decreases with smooth muscle tone is that smooth muscle tone causes an increase in RV from an increase in trapping. There was a significant negative correlation between the %ΔFVC and %ΔRV (r = −0.51, P = 0.018), but this degree of correlation was less than that between %ΔFVC and the FRC<sub>ratio</sub> (r = 0.58, P = 0.0065, Table 3). Furthermore, with stepwise regression of both %ΔRV and the FRC<sub>ratio</sub> as independent variables against %ΔFVC as the dependent variable, the %ΔRV became non-significant, leaving only the FRC<sub>ratio</sub> as a significant determinant of %ΔFVC. The implication of these relationships is that, whatever the mechanism of the increase in RV, and we shall later explore what that mechanism is, the effect of the BSMT on the FVC was more through how much the FRC increased relative to the increase in RV than through an independent effect of the increase in RV itself. Because of the very strong correlation between ΔFRC and ΔTLC (see above), we could hypothesize that the effect of BSMT on FVC was also determined by the TLC<sub>ratio</sub>. Indeed, the TLC<sub>ratio</sub> was a significant determinant of %ΔFVC (r = 0.65, P = 0.001).

Thus the increase in RV was necessary but not sufficient for the decrease in FVC with BSMT. For the FVC to decrease with
BSMT, the TLC must not have increased enough from an increase in FRC to prevent the decrease in FVC. Although the log10PC20 was significantly correlated with both %ΔFEV1 (Fig. 5) and the FRCratio (Fig. 6, Table 3), multiple stepwise regression showed that only the FRCratio was a significant determinant of log10PC20, suggesting that the magnitude of the response to both the BSMT and inhaled methacholine was significantly determined by how much the FRC (and, therefore, TLC) increased relative to the increase in RV.

The relationship between the FRCratio and airway dimensions. We examined the relationships between the FRCratio and the airway measurements for the three sizes of airways, with BSMT and after albuterol: 1) the luminal diameter at TLC, 2) the wall thickness at FRC, 3) the %Δ in the luminal diameter between BSMT and albuterol [18 total airway measurements for determination of the correlation coefficients (r) with the FRCratio]. The only significant correlations were between the luminal diameters at TLC of the large airways, both with BSMT and albuterol [DiTLCab(large), r = 0.60, P = 0.0190 and DiTLCals(large), r = 0.70, P = 0.0036]. With stepwise multiple regression, DiTLCbsm(large) was not significant, leaving only the correlation between the FRCratio and DiTLCab(large) as significant. DiTLCbsm(large) was not significant, because its effect was largely redundant through its tight correlation with DiTLCab(large) (r = 0.92, P < 0.0001).

At all values of DiTLCab(large) that were <13 mm, FRCratio was <1 (the increase in FRC was less than the increase in RV with the BSMT); at all values of DiTLCab(large) that were >13 mm, the FRCratio was ≥1 (Fig. 7). The significant correlation between FRCratio and DiTLCab(large) occurred only in the range where DiTLCab(large) was <13 mm and the FRCratio was <1 (r = 0.71, P < 0.05). In the range where DiTLCab(large) was >13 mm and the FRCratio was ≥1, there was no apparent correlation between the FRCratio and DiTLCab(large) (r = 0.14, P = 0.8). Thus the ability of the BSMT to decrease the FVC and FEV1 through a low FRCratio appeared to require a critical narrowing of the large airways in the absence of tone. When the luminal diameter of the large airways without tone was above this critical diameter (critical Diam), BSMT had little effect on the FVC and FEV1.

There was a significant correlation (r = 0.71, P = 0.003) between (FEV1/FVC)ab and DiTLCab(large), indicating that (FEV1/FVC)ab was a reasonable surrogate for large airway luminal narrowing. Accordingly, (FEV1/FVC)ab could be substituted for DiTLCab(large) as a significant determinant of %ΔFVC (r = 0.73, P = 0.0002), FRCratio (r = 0.54, P = 0.01), and TLCratio (r = 0.64, P = 0.002). There was no significant correlation between either DiTLCsm(large) or DiTLCsm(medium) and (FEV1/FVC)ab (P = 0.54 and 0.57, respectively).

The correlates of the response to a methacholine challenge. The observation that the log10PC20 of a methacholine challenge was significantly correlated with the magnitude of the reduction in FEV1 from the BSMT lends support to the idea that the reduction in FEV1 from either BSMT or a methacholine challenge involves similar mechanisms. The observation that both the log10PC20 and the reduction in FEV1 from BSMT were significantly correlated with the FRCratio (Table 3) suggests that the magnitude of the response to either BSMT or a methacholine challenge does not depend simply on how the smooth muscle tone affects the FEV1 directly, but also how much the FRC and therefore the TLC increases relative to the increase in RV with an increase in smooth muscle tone. The question that remains to be answered is why the FRCratio is positively correlated with the diameter of the large airways [DiTLCab(large)].

The effects of a diminished diameter of the large airways in the absence of smooth muscle tone [DiTLCab(large)], as reflected by a diameter <13 mm (critical Diam), was an increase in FRCab%, TLCab%, and FVCab%. This can be seen by partitioning these parameters by the critical Diam. One-way ANOVA revealed that FRCab%, TLCab%, and FVCab% were significantly increased in volunteers with a critical Diam of <13 mm (P = 0.0004, 0.02, and 0.01, respectively). Narrowing of the large airways was also associated with a decrease in FEV1ab/FVCab, that is compatible with an increased expiratory resistance. The mechanism of the association of increased lung volumes and narrowing of the large airways in the absence of smooth muscle tone is not clear, but dynamic hyperinflation or intrinsic positive end-expiratory pressure is likely playing a role.

The greater the FRCab%, TLCab%, and FVCab% before the BSMT, the greater was the responsiveness to the methacholine challenge (Table 4). One can infer from the relationships between the FRCratio and log10PC20 (Fig. 6) and between the FRCratio and TLCratio (Fig. 4) that the significant correlations between the lung volumes and the log10PC20 (Table 4) indicate that the greater the lung volumes were before the addition of smooth muscle tone, the less the TLC was able to increase for a given increase in RV from the challenge.

Table 4. Multivariate pairwise correlations of lung volumes in absence of baseline smooth muscle tone with log10PC20

<table>
<thead>
<tr>
<th>Variable by Variable</th>
<th>Correlation</th>
<th>Count</th>
<th>Significant Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRCab%</td>
<td>log10PC20</td>
<td>−0.54</td>
<td>14</td>
</tr>
<tr>
<td>TLCab%</td>
<td>log10PC20</td>
<td>−0.65</td>
<td>14</td>
</tr>
<tr>
<td>FVCab%</td>
<td>log10PC20</td>
<td>−0.65</td>
<td>14</td>
</tr>
<tr>
<td>FEV1/FVCab%</td>
<td>log10PC20</td>
<td>0.56</td>
<td>14</td>
</tr>
<tr>
<td>FRCratio</td>
<td>log10PC20</td>
<td>0.66</td>
<td>14</td>
</tr>
</tbody>
</table>

Ab, Albuterol.
The responses to both the BSMT and the methacholine challenge appear to be affected by how much the TLC increases in relation to the increase in RV, and that relative increase, in turn, is determined by the FRC ratio (Fig. 4). The FRC ratio decreases as the diameter of the large airways is narrowed, because the FRC is already elevated before the increase in smooth muscle tone, perhaps from dynamic hyperinflation, and that likely attenuates the degree of increase in the FRC when the smooth muscle tone is increased.

The relationship between the ΔRV and the airway dimensions. As in the analysis of the FRC ratio and airway dimensions, we analyzed the correlation coefficients between the same 18 airway dimensions and ΔRV. There were five significant correlation coefficients: \( \Delta \text{DiTLC}_{\text{abl}} \text{(medium)} \) \( r = -0.46, P = 0.035 \); \( \Delta \text{DiTLC}_{\text{abl}} \text{(large)} \) \( r = -0.56, P = 0.0312 \); \( \text{WthFRC}_{\text{bsmt}} \text{(large)} \) \( r = 0.65, P = 0.0083 \); and \( \text{WthFRC}_{\text{abl}} \text{(large)} \) \( r = 0.64, P = 0.0100 \). With backward stepwise regression, there were only two significant determinants of ΔRV, accounting for nearly 70% of the variance: \( r^2 = 0.68, P = 0.0011 \); \( \text{DiTLC}_{\text{abl}} \text{(medium)} \), \( F \) ratio = 9.89, \( P = 0.0085 \); \( \text{WthFRC}_{\text{abl}} \text{(large)} \), \( F \) ratio = 18.26, \( P = 0.0011 \).

Since the luminal diameter of the large airways \( \text{DiTLC}_{\text{abl}} \text{(large)} \) was the determinant of the FRC ratio that quantified how much the FRC increased in relation to the increase in RV with BSMT, the addition of \( \text{DiTLC}_{\text{abl}} \text{(large)} \) to the two determinants of the increase in RV \( \text{DiTLC}_{\text{abl}} \text{(medium)} \) and \( \text{WthFRC}_{\text{bsmt}} \text{(large)} \) should be a major determinant of the increase in FRC (ΔFRC). Indeed, the addition of \( \text{DiTLC}_{\text{abl}} \text{(large)} \) to the determinants of RV accounted for nearly 90% of the variance on the ΔFRC: \( r^2 = 0.88, P < 0.0001 \); \( \text{DiTLC}_{\text{abl}} \text{(medium)} \), \( F \) ratio = 59.21, \( P < 0.0001 \); \( \text{WthFRC}_{\text{abl}} \text{(large)} \), \( F \) ratio = 37.88, \( P < 0.0001 \); \( \text{DiTLC}_{\text{abl}} \text{(large)} \), \( F \) ratio = 14.55, \( P = 0.0029 \).

The BSMT produced a significant decrease in the luminal diameter at TLC in all three sizes of airways (Table 5). There were no significant changes in the WthFRC of any sized airway with BSMT. There was no significant correlation between the decrease in the diameter of any sized airway and the increase in the RV with the BSMT.

Summary of effects of the response to BSMT and methacholine challenge. The BSMT principally affected the baseline pulmonary function through an increase in RV that correlated with parameters that could be highly influenced by airway remodeling, such as the wall thickness of the large airways and the relaxed luminal diameter of the medium airways. The effect of this increase in RV on the pulmonary function parameters was dependent on the presence or absence of a critical luminal narrowing of the large airways measured in the absence of BSMT. Specifically, if the relaxed large airway luminal diameter exceeded this critical value, the increase in RV was accompanied by an increase in FRC and TLC that was essentially as great as the increase in RV, so that there was little effect of the BSMT on the FVC. At large airway diameters below the critical value, the degree of narrowing of the lumen of the large airways caused a progressive decrease in the magnitude of the increase in TLC relative to the increase in RV with the BSMT, so that there was a progressive decrease in the FVC. An increase in the wall thickness of the large airways or a decrease in the luminal diameter of the medium airways caused an increase in RV, but also an increase in TLC to the same extent as the increase in RV, such that the FVC was preserved. Below the critical luminal diameter of the large airways, the already increased FRC, presumably from dynamic hyperinflation, attenuated the increase in FRC and TLC with BSMT. All of the major findings of this study were compatible with the hypothesis that the magnitude of dynamic hyperinflation from a decrease in the luminal diameter of the large airways was the most important determinant of the pulmonary function under conditions of BSMT.

This study evaluated pulmonary function and airway structure on 2 separate days: on 1 day, under baseline conditions, and on another day, after a maximum response to albuterol. We made the assumption that the difference in pulmonary function between the 2 days was caused by the BSMT that had been abolished by albuterol. We assumed that the change in pulmonary function caused by albuterol is due only to the abolition of the bronchial smooth muscle tone (32). We defined the magnitude of the difference in pulmonary function between the 2 days as the response to BSMT. We used the magnitude of the response to BSMT in relation to airway dimensions determined by HRCT to make inferences concerning the relationship between airways structure (remodeling) and AHR in volunteers with moderate to severe asthma.

| Table 5. Change in airway luminal diameter produced by the baseline smooth muscle tone |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Units                           | Albuterol       | BSMT            | %Δ               | Significant Probability |
| DiTLC(large) mm                 | 13.36±0.31      | 12.86±0.37      | −3.8±1.1         | 0.003            |
| DiTLC(medium) mm                | 8.21±0.14       | 7.34±0.09       | −10.5±1.0        | <0.0001          |
| DiTLC(small) mm                 | 5.68±0.10       | 4.83±0.08       | −14.7±0.1        | <0.0001          |

Values are means ± SE. BSMT, baseline smooth muscle tone; DiTLC(large), DiTLC(medium), and DiTLC(small): large, medium, and small airway diameter measured at TLC, respectively.
To understand the relationship between airway structure and the severity of asthma, we need to understand how airway structure was related to 1) the mechanism of the increase in RV with BSMT, and 2) the mechanism of the increase in TLC with BSMT.

Before proceeding further, several potential limitations to these measurements should be considered. First, the spirometry and lung volume measurements were determined in the upright position, whereas HRCT was performed supine. There is an ~25% decrease in FRC in the supine position (25), associated with an increase in airway resistance (31). We have shown this to occur in previous HRCT studies from our laboratory (4).

Given that this phenomenon occurred in all volunteers in the present study, we do not think it affected the validity of our comparisons between patients. A second potential limitation of our study was the inability of HRCT to visualize and measure very small airways below the resolution of the CT scanner. While we have difficulty visualizing and measuring airways <1 mm in diameter with our current CT technology, recent data from Nakano et al. (30) demonstrate the utility of conducting airways to reflect the changes in the respiratory airways. Finally, we assumed that we could eliminate smooth muscle tone with albuterol, despite the fact that airway smooth muscle contraction in asthma is mediated by a variety of mechanisms. Thus it is possible that complete smooth muscle relaxation was not achieved with high-dose albuterol alone.

Despite this potential limitation, we were able to observe significant changes in airway luminal diameter with albuterol (Table 5) and thus consider it reasonable to assume that the significant changes in airway luminal diameter with albuterol relaxation was not achieved with high-dose albuterol alone. Nevertheless, the findings are exactly those that would be predicted from a theoretical analysis of how smooth muscle tension in blood vessels or airways produces a critical closing pressure (9, 34, 35). If the elastic recoil pressure (airway pressure relative to pleural pressure under static conditions) is considered the distending or transmural pressure (Ptm) of the airways, then the critical closing pressure of the airways in the presence of smooth muscle tone is the Ptm at airway closure, or Ptm’ (15, 35). An increase in Ptm’ causes the RV to increase because of airway closure. The theoretical analysis of how smooth muscle tone causes a critical closing pressure showed that the magnitude of Ptm’ was determined by the ratio of the smooth muscle tension to the radius of the unstressed lumen in the absence of smooth muscle tension (9, 34). This phenomenon was recently demonstrated to be the predominant mechanism of increased respiratory impedance in the antigen-challenged, sensitized mouse lung (43).

The fact that only the luminal diameter of the medium airways and the wall thickness of the large airways at TLC in the absence of the smooth muscle tone were determinants of the change in RV produced by smooth muscle tone could explain why there has been so little evidence of an association between the luminal diameter of the airways and pulmonary function measurements (6). The relationships between the responses to smooth muscle tone and airway structure have not been previously studied with the airways at TLC in the absence of smooth muscle tone.

We did not measure the change in the luminal diameters with increasing doses of a muscle spasmogen, but we have evidence from other studies that the greater the activation of the airway smooth muscle, the greater the decrease in the luminal diameter of the airways (4). Since we found no correlation between the magnitude of the change in pulmonary function and the change in the luminal diameter of the airways that was produced by the BSMT in the present study, it became plausible that there was purely a structural basis to the variance of the effect of BSMT on pulmonary function. These findings are compatible with a recent study of Schueller et al. (38), who found that the difference in airway luminal area between baseline and postalbuterol or between baseline and histamine challenge was the same for three groups: a group of healthy volunteers and two groups of asthmatic subjects, those with or without a significant decrease in FEV1 with the histamine challenge.

In the present study, there was no evidence that closure of the measured small airways played a significant role in the effect of BSMT on RV. The results of the present study are compatible with other studies on the role of closure of large airways. Even large, cartilaginous airways can completely close from smooth muscle contraction (5, 28). Moreover, there is evidence that small airways respond less to cholinergic stimulation than large airways (10, 29). Nevertheless, the luminal narrowing in the visualized airways in the present study could also be a marker for functionally important unvisualized changes in the distal lung (30). In addition, modeling studies have suggested that neither small nor large airway closure alone can explain the ventilation defects observed on PET scanning associated with bronchoconstriction in asthmatic subjects (42).

The mechanism of the increase in TLC with BSMT. The increase in TLC was closely related to the increase in FRC (Figs. 3A and 4). Without an increase in FRC, there was little increase in TLC. Perhaps an increase in FRC produced by the BSMT is somehow sensed by the central nervous system, leading to a greater neural drive to the inspiratory muscles, resulting in an increased TLC. As lung volume is increased from a voluntary effort, the neural drive to the inspiratory muscles of the thorax continuously increases the outward retractive force of the thorax, acting in opposition to the continuously increasing inward retractive force of the lungs (the elastic recoil of the lungs) until they are balanced at TLC (15, 27). A greater neural drive to the inspiratory muscles would cause the balance between the outward force of the thorax and the inward force of the lungs to be reached at a higher TLC.

In some studies, during the recovery from acute asthma or during challenge of asthmatic patients with spasmsogens, an increase in TLC has been observed (13, 16), but not invariably (24). Martin et al. (26) suggested that there was increased activity of the inspiratory muscles in proportion to the increase in FRC during inhaled histamine challenge in seven asym-
matic asthmatic subjects. In three of the seven volunteers, there was an increase in TLC by 10, 12, and 22% over the baseline TLC. The authors suggested that increased muscle action was a major determinant of the degree of hyperinflation. The increased neural drive suggested by Martin et al. (26) was directly observed in a study of the electromyographic activity of the diaphragm in asymptomatic, asthmatic patients with an increase in FRC produced by histamine challenge (20). Pernick et al. (33) examined respiratory mechanics during an induced attack of acute asthma from exercise in a volunteer who was trained to carry out the respiratory maneuvers required to measure the maximum outward retractive force of the thorax at TLC. There was an increase in RV, FRC, and TLC during the attack, accompanied by a significant increase in the outward retractive force of the thorax at TLC that the authors attributed only partly to an increase in inspiratory muscle contractility. In addition to the increased contractility of the inspiratory muscles, the authors suggested that part of the increase in outward force was caused by a shift of the pressure-volume (PV) curve of the chest wall that they inferred was from a change in the intrinsic elastic properties of the chest wall. Martin et al. (26) found a similar shift in the PV curve of the relaxed chest wall.

In addition to the shift in the PV curve of the thorax (26, 33), shifts in the PV curve of the lungs have been observed where there has been a decrease in the elastic recoil pressure at the same volume and an increase in the expiratory compliance (13, 16, 17). A primary shift in the PV curves of the lungs has been suggested as the cause for the increase in the TLC (19), but the changes in the PV characteristics are exactly opposite to what was found in a study of submaximal neuromuscular blockade in healthy volunteers (14). Thus the change in the static PV characteristics of the lungs and thorax that accompanies the increase in TLC would appear to be more likely the result of the increase in the ability of the inspiratory muscles to expand the thorax. The PV characteristics of excised lungs change in a nearly identical manner to those found during an asthmatic attack, with nothing more than an increase in the end-inspiratory pressure and volume to a new level (18). These changes are caused by the marked effect of volume history on the PV relationship (18, 21).

On the basis of the evidence that increased inspiratory muscle contractility and increased FRC go together in asthma, we infer that the increase in TLC with BSMT or acute asthma is the result of a greater neural drive to the inspiratory muscles that is a response to the increase in FRC.

Comparisons of the responses of RV, TLC, and FVC to the BSMT. The response to the BSMT in terms of the magnitude of the mean absolute increase in RV and the decrease in FVC for the 21 volunteers was 0.91 and 0.27 liters, respectively (Table 2, Fig. 1). The mean increase in TLC was 0.63 liters, exactly equal to the difference between the RV and FVC response (Fig. 1). If there had been no increase in TLC, the decrease in FVC would have been equal to the increase in RV, but the measured mean increase in RV was 3.3 times greater than the decrease in FVC; i.e., the decrease in FVC with BSMT would have been more than threefold greater, if there had been no increase in TLC with the BSMT.

The results of the present study are not compatible with the conventional interpretation of the role of TLC in asthma, where it is assumed that increases in TLC occur only infrequently and in the most severe asthmatic attacks. On the contrary, the change in FVC and FEV₁ appears to be the tip of the iceberg of a much greater change in RV and TLC in the typical response to an increase in smooth muscle tension, i.e., where there is no luminal narrowing of the large airways. However, when the lumen of the large airways is sufficiently narrowed to lead to dynamic hyperinflation, the increase in smooth muscle tension leads to an increase in RV without a compensatory increase in TLC, thus causing the FVC to fall. In support of this hypothesis, Carroll et al. (11) compared airway remodeling between fatal and nonfatal cases of asthma. They found more remodeling of the large airways in the fatal than nonfatal cases of asthma, but there were no differences between the two groups in the remodeling of the small airways. The relationship between large airway remodeling and asthma severity in this study could be explained, at least in part, by the diminished compensation of an increase in TLC with smooth muscle contraction when there is a narrowing of the lumen of the large airways.

A healthy person has no narrowing of the lumen of the large airways and is able to tolerate a very high dose of a contractile agonist with little decrease in FVC or FEV₁. The results of the present study suggest that this apparent hyporesponsiveness should be accompanied by a considerable increase in RV, but a simultaneous increase in TLC suppresses the change in FVC and FEV₁. At this point, this postulate has not been experimentally tested. If the challenge with the contractile agonist were carried out with suppression of deep inspirations in healthy volunteers, as in the study of Skloot et al. (39), it is possible that the expected increase in TLC accompanying the increase in RV could be suppressed. If this is so, an alternative interpretation of the results of the study of Skloot et al. in healthy volunteers could be made: the suppression of deep inspirations during the methacholine challenge markedly increased the response, not because the suppression of deep inspirations caused a greater increase in RV, but, rather, the suppression prevented the compensatory increase in TLC.

The role of (FEV₁/FVC)lab in the structural basis of AHR. The results from the present study suggest that the major structural basis of the AHR, as estimated by the effect of BSMT, resulted from a narrowed caliber of the fully relaxed large airways that attenuated the increase in TLC with smooth muscle constriction. A conventional pulmonary function measurement in the absence of smooth muscle tone, (FEV₁/FVC)lab can be used as a surrogate for DiTLC lab(large). Indeed, (FEV₁/FVC)lab was significantly correlated with the same indexes as the DiTLC lab(large). In support of the special significance of FEV₁/FVC, as a reflection of the caliber of the large airways in relation to AHR in asthma, Britton et al. (3) found that FEV₁/FVC was a determinant of AHR in asthma that was independent of atopy and even FEV₁. Bacharier et al. (2) studied the relationship between asthma severity, judged by symptoms and medication use, and pulmonary function in children. They found that FEV₁/FVC, but not FEV₁, decreased as asthma severity increased.

The longitudinal study of Rasmussen and his colleagues (36) used (FEV₁/FVC)lab, as an index of airway remodeling without consideration of its special relationship to remodeling of the large airways. Their study showed that subjects with a reduced (FEV₁/FVC)lab, at 18 or 26 yr old were more likely to have asthma, AHR, and an increased probability of having had the same characteristics throughout childhood. The link found in the present study between large airway dysfunction, AHR, and severity, similar to the findings in the study by Rasmussen et al. (36), raises the possibility that the stage is set in early childhood for significant AHR being caused by some chronic insult.
to the large airways that is superimposed on, and independent of, the more typical characteristics of asthma.

In summary, the findings of this study suggest that the magnitude of the hyperresponsiveness in asthma is a function of the intrinsic structure of the airways. Increases in the wall thickness of the large airways or decreases in the luminal diameter of the medium airways were associated with increases in RV. The increase in RV is normally accompanied by an increase in TLC that occurs with obstruction in vivo during lung vascular engorgement and edema. Am J Respir Crit Care Med 156: 787–793, 1997.


