Compliance, Hysteresis, and Collapsibility of Human Small Airways

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We tested the hypothesis that airway wall dimensions are important determinants for the mechanical properties of airways. Lung tissue was obtained from 31 smokers with different degrees of chronic obstructive pulmonary disease (COPD) who were operated on for a solitary lung lesion. Segments of small airways (n = 35) were mounted on cannulas in an organ bath and inflated and deflated cyclically between +15 and −15 cm H2O. For each airway this was done at baseline, after methacholine, and after isoprenaline. Specific compliance (sCdyn), specific hysteresis (sHs), and pressure at which the airways collapsed (Pcol) were calculated from each recording. Airway wall dimensions were measured morphometrically. Lung function parameters of airflow obstruction were correlated to sCdyn, sHs, and Pcol. At baseline, after methacholine, and after isoprenaline sCdyn was 0.059, 0.052, and 0.085 cm H2O−1, sHs was 13.5, 12.9, and 7.1%, and Pcol was −3.4, −3.5, and −1.9 cm H2O, respectively. Differences between sCdyn, sHs, and Pcol after methacholine and after isoprenaline were highly significant (p < 0.001). Of all dimensions studied, smooth muscle area, but not total wall area, was the most important determinant for sCdyn and for sHs after methacholine. Specific hysteresis at baseline correlated to residual volume as a fraction of total lung capacity (RV/TLC) (r = 0.5, p = 0.05) and, in the presence of methacholine, to FEV1/FVC (r = −0.68, p = 0.02) and RV/TLC (r = 0.5, p = 0.05). We conclude that, in this study, smooth muscle area and smooth muscle tone, but not total wall area, are determinants for compliance, hysteresis, and collapsibility of isolated airways obtained from smokers. Tiddens HAWM, Hofhuis W, Bogaard JM, Hop WCJ, de Bruin H, Willem's LNA, de Jongste JC. Compliance, hysteresis, and collapsibility of human small airways.

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In chronic obstructive pulmonary disease (COPD) airway inflammation is associated with increased airway wall thickness (1). This thickness is relatively more severe in peripheral airways than in central airways, and is more pronounced in patients with more severe airflow obstruction (1). Similar findings were obtained in patients who died with or from asthma (2, 3). Thickening of the airway wall as such has little effect on airway resistance (1). However, in combination with smooth muscle shortening it causes an important increase of airway resistance (4, 5). The extent to which the smooth muscle in airways of patients with COPD shortens will depend on the force it develops and on the load against which the smooth muscle has to contract. Increased smooth muscle force in COPD has been described by some investigators (6) but not by others (7). Isotonic shortening was decreased in airway muscle from patients with COPD compared with smokers without airflow obstruction (8). These findings suggest that in COPD the load on the bronchial smooth muscle might be increased. In fact, chronic airway inflammation is associated with deposition of fibrous tissue such as collagen and elastin within different compartments of the airway wall (9–13). This probably makes airways stiffer (i.e., less compliant) and increases the load against which the smooth muscle contracts. We hypothesize that airway wall thickness is an important determinant of the mechanical properties of airways and that reduced compliance makes the airways less collapsible and decreases their distensibility and hysteresis.

This study was undertaken to evaluate the relation between compliance, collapsibility, and hysteresis, on the one hand, and wall dimensions of human peripheral airway segments on the other hand. Therefore, we have developed an experimental model to study compliance, hysteresis, and collapsibility of isolated human small airway segments at baseline tone and after maximal contraction and relaxation of the airway smooth muscle.

METHODS

Study Population

Lung tissue was obtained from 31 patients who had a lobar resection or pneumonectomy for a peripheral lung lesion. These patients were...
mostly smokers and were classified as "COPD" although one-third had a lung function within the normal range and, therefore, did not strictly fulfill the American Thoracic Society criteria (14). Preoperative routine lung function was obtained from 27 of 31 patients. Forced expiratory volume in one second (FEV$_1$), forced vital capacity (FVC), maximal flows at 50% ($\text{V} \times 50\%)$ and 25% ($\text{V} \times 25\%)$ of FVC, functional residual capacity (FRC), total lung capacity (TLC), residual volume (RV), and RV as a fraction of TLC (RV/TLC% pred) are all expressed as percentage of predicted (15). We used FEV$_1$, expressed as the absolute change in FEV$_1$ as a percentage of FVC (FEV$_1$/FVC), as an indicator of the severity of airflow obstruction. Reversibility of bronchial obstruction was expressed as the absolute change in FEV$_1$ as a percentage of the predicted FEV$_1$ (ΔFEV$_1$% pred) and as a percentage of the actual pre-bronchodilator FEV$_1$ (ΔFEV$_1$% ini). This protocol was approved by the institutional review board for human studies.

Airway Segments

Lung tissue was collected and transported on ice in carbogenated (5% CO$_2$, 95% O$_2$) Krebs-Henseleit buffer (composition in mM: NaCl 118, KCl 4.7, CaCl$_2$ 2.5, MgSO$_4$ 1.2, KH$_2$PO$_4$ 1.2, NaHCO$_3$ 25, glucose 5.55). Peripheral airways were identified, cannulated with a thin plastic cannula, and dissected out with surrounding parenchyma. The airways were stored overnight in carbogenated Krebs-Henseleit buffer at 4°C. Next morning, parenchyma and blood vessels were removed with the help of a dissection microscope (Stemi 2000; Carl Zeiss, Oberkochen, Germany) and all side branches identified and ligated in a watertight fashion. Airway segments at least 5 mm in length were selected for in vitro measurements. The internal diameters of the endings of the airways were estimated using the microscope by selecting stainless steel cannulas (1, 1.5, 2, 3, or 4 mm) that most closely fitted the diameter of the airway. Next, the airways were mounted in the organ bath on the cannulas in a watertight fashion. The airways were perfused to test for patency and possible leaks. The length of the unstretched airway within the inner borders of the cannula sutures was measured by a digital calipers (precision 0.1 mm). Next, we stretched the airway to 140% of this length, that being the estimated length of an airway close to TLC (16). At this length the airway remained patent at low transmural pressures and after contraction by methacholine. The volume (µl) of the airway was estimated by the formula $\pi r^2 l$, where $r$ is the average of the radius (mm) of the two cannulas and $l$ is the length (mm) of the stretched airway.

Description of Experimental Setup

Figure 1 shows the experimental setup we used to measure the dynamic properties of the airway segments. It consists of a double-jack- eted organ bath in which the cannulated airway segment was mounted. The organ bath is filled with Krebs-Henseleit buffer at a temperature of 37°C and continuously gassed with carbogen. A airway segment and tubing are filled with buffer. A II connecting tubing has a low compliance. Pressure was measured at both ends of the airway segment ($P_{\text{prox}}$ and $P_{\text{dist}}$) using pressure transducers with a low compliance (143 PC ± 1 psi; Micro Switch, Freeport, IL). The compliance of the whole system was 0.03 µl cm H$_2$O$^{-1}$. By means of a 3-way valve, the airway segment can be connected to a gas-tight syringe mounted on a computer-controlled, high-precision syringe pump (Harvard Apparatus Inc., South Natick, MA) for cyclic inflation and deflation. Furthermore, the segment can be connected to a microplethysmograph that was designed to measure small volume displacements from or to the airway under isobaric conditions. Finally, the segment can be connected to a reservoir filled with buffer for perfusion. The transmural pressure signals ($P_{\text{prox}}$ and $P_{\text{dist}}$), the volume signal of the syringe pump, and the volume signal of the microplethysmograph ($P_{\text{diff}}$) are all displayed on the computer monitor. The system is servo-controlled, with the pressure signal from $P_{\text{prox}}$ being used to control the syringe pump.

**Figure 1.** Diagram of the experimental setup to measure dynamic properties of isolated airway segments. A = cannulated airway; $V_{\text{prox}}$ and $V_{\text{dist}}$ = valves located at the proximal or distal end of the airway; $V_{\text{pump}}$ = valve to connect pump to airway; $P_{\text{prox}}$ and $P_{\text{dist}}$ = pressure transducers to measure the transmural pressure at the proximal and distal ends of the airway; microplethysmograph = device to measure small volume displacements under isobaric conditions; multichannel registration = system for processing of pressure and volume signals; high-pressure pump = pump for cyclic inflation and deflation of airway. The system is servo-controlled, with the pressure signal from $P_{\text{prox}}$ controlling the syringe pump.
at 8 times the estimated volume of the airway segment per minute. From a pilot study, we estimated that this would give a cycling rate between 1 and 4 cycles per minute.

Leak test. To test for a leak, the airway was inflated by the pump until a transmural pressure of 15 cm H₂O was obtained. Next, the spontaneous rate of pressure drop of the segment within the first minute of inflation was recorded. In case of leakage it was either impossible to inflate the airway to 15 cm H₂O, or its transmural pressure dropped rapidly to 0 cm H₂O after inflation stopped.

Pressure-volume relation at baseline. Zero pressure of the proximal and distal valves was set by connecting the airway lumen to the atmosphere. The airway was then connected to the pump. Next, the airway was cyclically inflated and deflated between +15 and −15 cm H₂O using the computerized servo-controlled pump (Figure 2). The upper pressure limit of 15 cm H₂O was chosen because it corresponds to the plateau of the pressure-volume curve of an air-filled system (17). This transmural pressure is, therefore, likely to inflate the airway to a level comparable to TLC. Three complete inflation-deflation cycles were done. After the third cycle, the airway was inflated to 15 cm H₂O at which point the pump automatically stopped. We recorded the spontaneous pressure drop within 2 min to estimate maximal hysteresis and leak.

Isobaric contractility and relaxation. To monitor the contractile response to methacholine or relaxation response to isoprenaline the airway was connected to the microplethysmograph. The transmural pressure was set at 10 cm H₂O by adjusting the height of the plethysmograph. Baseline volume displacement out of the plethysmograph was recorded for 2 min before sufficient methacholine or isoprenaline was added to the organ bath to give a final concentration of 10⁻² M. The isobaric constriction or relaxation was recorded until no more fluid was displaced into the plethysmograph or until the rate of fluid displacement out of the plethysmograph became constant.

Pressure-volume relation in the presence of methacholine and isoprenaline. These were measured as described for baseline tone.

Isobaric relaxation. To monitor the relaxation response to isoprenaline the airway was connected to the microplethysmograph. The transmural pressure was set at 10 cm H₂O by adjusting the height of the plethysmograph. Baseline volume displacement out of the plethysmograph was recorded for 2 min before sufficient methacholine or isoprenaline was added to the organ bath to give a final concentration of 10⁻² M. The isobaric relaxation was recorded until the rate of fluid displacement out of the plethysmograph became constant.

Pressure-volume relation after isoprenaline. This was measured as described for baseline.

Fixation. Finally, the airway was perfused with formalin (10%) from the distal end at a driving pressure of 10 cm H₂O. The valve was closed as soon as formalin came out of the proximal valve and the Krebs buffer in the organ bath was quickly replaced with formalin. A filter fixation for 12 h at a transmural pressure of 10 cm H₂O, the airway, including the ligatures at both ends, was gently pushed off the cannulas with forceps and stored in formalin until being processed for histology.

Compliance, Hysteresis, Collapsibility, and Contractility

Compliance (Cdyn, µl cm H₂O⁻¹) was calculated from the volume infused to inflate the airway from 0 to 15 cm H₂O (Figure 2). The transition point of the pressure-volume curve where inflation changes to deflation was selected as the upper pressure limit for the Cdyn calculation because it is least affected by hysteresis. Furthermore, it results in the Cdyn of the stretched airway close to TLC where collagen is likely to be an important mechanical determinant (18, 19). Therefore, Cdyn should be sensitive to changes in airway collagen content. Hysteresis (h, µl cm H₂O) was calculated as the area of the pressure-volume loop between 0 and 15 cm H₂O. Collapsibility (Pcol, cm H₂O) was defined as the pressure of the deflation limb where the distal pressure did not follow the proximal pressure, thus indicating closure of the airway lumen. Cdyn, h, and Pcol were calculated for each cycle. We analyzed Cdyn, h, and Pcol of the first cycle (Cdyn₁, h₁, and Pcol₁) separately because we observed that these were always substantially different from those of the subsequent cycles. The latter were similar and were therefore taken together (Cdyn, h, and Pcol). The first cycle was excluded for analysis when it did not start exactly from 0 cm H₂O transmural pressure. Volume loops could not be analyzed for hysteresis in 11 airways where we used a high syringe volume and low speed of the pump as this caused artifacts in the pressure-volume loops.

To normalize compliance for the volume of the airway segment, Cdyn₁ and Cdyn were divided by the volume of the airway segment as calculated by morphometry (sCdyn₁, sCdyn). Hysteresis was normalized as a percentage of the area of the maximal possible hysteresis (s₁, s), which was defined as the product of volume infused and the upper pressure limit (15 cm H₂O). The spontaneous pressure drop within 2 min after the last inflation was normalized for the inner surface area of the airway (internal perimeter times length) (specific pressure drop, cm H₂O mm⁻²). We calculated the maximal volume displaced by the airway segment after methacholine (Vₘ), or isoprenaline (Vᵢ). The response was negative when fluid moved out of the contracting segment into the plethysmograph and positive when fluid moved into the relaxing airway segment out of the plethysmograph. The response was corrected for the rate of baseline fluid movement before methacholine or isoprenaline was added. To correct for the volume of the airway segment, Vₘ and Vᵢ were expressed as the percentage of the volume of the airway segment as calculated from morphometry.

Airway Dimensions

To estimate airway wall dimensions and volume of the lumen, the segment was cut in cross section at both ends just inside the ligatures.

Figure 2. Example of pressure-volume tracing. The arrowhead shows the direction of the loop. Dynamic compliance (Cdyn) is the division of infused volume by 15 cm H₂O. Specific hysteresis is the area of the pressure-volume loop above 0 cm H₂O expressed as the percentage of the area of the maximal hysteresis.
decataloged, and embedded in paraffin in such a way that the airway segment could be cut in cross-section. A random starting point was selected within the first 0.5 mm at one end of the airway using a randomization table. Sections of 5 μm thickness were cut all the way through the airway with a microtome. Every 100th section (0.5 mm) was selected for morphometric analysis. We estimated that with this procedure we could obtain a reliable estimate of the mean airway dimensions. Sections were stained with a combined Gomori trichrome and elastin stain. This stain resulted in a good color contrast between airway wall structures. The measurements made are shown in Figure 3 and include: inner perimeter (P1i) and area of the lumen bounded by the respiratory epithelium (A1); basement membrane perimeter (Pbm) and the area enclosed by this perimeter (A1m); the outer muscle perimeter (Pmo) and the area enclosed by this perimeter (A1o); the outer perimeter (P5) and the area enclosed by this perimeter (A5); the area occupied by smooth muscle (WA); and the area occupied by cartilage (WAcart).

Furthermore, we measured the height of the respiratory epithelium (Hepi) and the fraction of the Pbm covered by epithelium (Fepi). Hepi was measured as follows. First, a grid containing parallel sinusoids was superimposed over the computer image of the airway. When respiratory epithelium was present at the point where the sinusoid crossed the basement membrane, we measured the epithelial height by drawing a straight line perpendicular from the membrane to the top of the ciliary border. The length of this line was automatically computed. When respiratory epithelium was absent, a zero was scored for height. Second, Hepi was calculated for each airway section by computing the average of at least 15 epithelial height measurements (100% × SEM)/mean. In our formula, SEM relates to the variation between sections within a single airway. The relationships between each airway wall dimension (WA, WAcart, WAo, WA1o, WA2, WA3, WA4, WA5, WAepi, Hepi, Fepi) and airway size (Pbm) were assessed using linear regression analysis. Previous studies found linear relationships between the square root of airway wall areas and airway size (1, 11, 23–25). We did a square root transformation on the airway areas of this study, again resulting in normal distributions of data.

We calculated the average specific compliance, specific hysteresis, and collapsibility for the first cycle (sCDyn1, sNH1, sPCol1) and for the average of the second, third, and fourth cycle (sCDyn, sNH, sPCol) for all airway segments. Furthermore, we calculated the average specific pressure drop. This was done for each of the three contractile conditions (baseline, methacholine, and isoprenaline). Repeated-measurements analysis of variance (ANOVA) was used to assess the overall differences between the three conditions, followed by pairwise t-tests in case the former test was significant. We investigated the correlation...
between the mechanical properties of the second, third, and fourth cycle (sCdyn, sη, Pcol) with airway wall dimensions (WA bm, WA p, WA m, WA cart, WA epil, P col) using Spearman’s correlation coefficient (r). When a mechanical property correlated to various airway wall dimensions in univariate analysis, we studied their simultaneous effects using multiple regression analysis. Data are expressed as mean ± standard deviation and range, unless indicated otherwise. p Values given are two-tailed. In view of the multiple comparisons, p values between 0.05 and 0.01 are considered as indicative only.

**RESULTS**

**Study Population and Lung Function**

Lung function and patient characteristics of the patients with COPD are shown in Table 1. Out of the group of 27 COPD patients with preoperative lung function test results, eight patients had no significant reduction of maximal expiratory airflow (FEV1/FVC > 70%), 12 patients had a mild reduction of maximal expiratory airflow (FEV1/FVC 60 to 70%), and seven patients had a more severe reduction (FEV1/FVC < 60% predicted). Increased residual volume (RV > RV pred + 2SD) was present in five of the 18 patients who had body plethysmography. Reversibility of bronchial obstruction defined as ΔFEV1% pred > 15% and ΔFEV1% ini > 15% was not present in any of the 23 patients.

**Airway Segments**

We successfully completed all measurements of 35 cannulated watertight airway segments. The diameter of the cannulas used was 1.3 ± 0.39 (1 to 2) mm; the length of the stretched airways was 14.2 ± 4.2 (6.7 to 21.2) mm. The median of the estimated volume of the segments was 30.7 (5.3 to 66.6) μl. Cycling frequency was 3.1 ± 1.9, 5 ± 5.4, and 1.8 ± 0.9 cycles min⁻¹ for baseline, methacholine, and isoprenaline, respectively. At baseline, after methacholine and isoprenaline, 13, 13, and 2 out of the cycles, respectively, started above 0 cm H2O and were therefore excluded for analysis.

**TABLE 2**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cycle 1 Meas ± SD (range)</th>
<th>Cycles 2, 3, and 4 Average Meas ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdyn, μl cm H2O⁻¹</td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>22</td>
<td>1.45 ± 1.0 (0.2 to 3.8)</td>
</tr>
<tr>
<td>Methacholine</td>
<td>22</td>
<td>1.36 ± 1.1 (0.1 to 3.6)</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>33</td>
<td>2.43 ± 1.5 (0.1 to 5.7)</td>
</tr>
<tr>
<td>sCdyn, cm H2O⁻¹</td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>22</td>
<td>0.053 ± 0.02 (0.02 to 0.09)</td>
</tr>
<tr>
<td>Methacholine</td>
<td>22</td>
<td>0.049 ± 0.03 (0.01 to 0.09)</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>33</td>
<td>0.080 ± 0.03 (0.01 to 0.15)</td>
</tr>
</tbody>
</table>

* Mechanical properties of isolated airway segments at baseline, after contraction with methacholine, and after relaxation with isoprenaline. The mechanical properties examined are compliance (Cdyn), hysteresis (η), and collapsibility (Pcol). Compliance was normalized for airway volume (sCdyn) and hysteresis for the maximal possible hysteresis (sη). The values for the first cycle and for the average of the consecutive cycles are shown separately because the mechanical properties of the first cycle were different from that of consecutive cycles.

**Compliance, Hysteresis, and Collapsibility**

The values for compliance and specific compliance (Cdyn and sCdyn), hysteresis and specific hysteresis (η and sη), and collapsibility (Pcol) at baseline, after methacholine, and after isoprenaline are shown in Table 2 and in Figure 4.

On average, the specific compliance of the first cycle (sCdyn) was 13, 11, and 8% below the mean sCdyn of the subsequent cycles for baseline (p < 0.001), methacholine (p = 0.03), and isoprenaline (p < 0.001), respectively (Figure 4). The mean sCdyn after methacholine was 0.009 cm H2O⁻¹ lower than that of baseline (p = 0.04). sCdyn after isoprenaline was 0.033 cm H2O⁻¹ higher compared with baseline (p < 0.001). The mean specific hysteresis after methacholine was 0.2 cm H2O⁻¹ higher than after baseline (p = 0.04), and 0.042 cm H2O⁻¹ higher compared with methacholine (p < 0.001). Expressed as percentages, these differences were 4, 101, and 197%, respectively.

The mean specific hysteresis of the first cycle was 34, 30, and 37% higher compared with sη of the subsequent cycles for baseline, methacholine, and isoprenaline, respectively (p < 0.001) (Figure 4). The mean sη after methacholine was not significantly different from baseline. sη after isoprenaline was 6.8% lower compared with baseline (p < 0.001) and 7% lower compared with methacholine (p < 0.001). Expressed as percentages, these differences were 9 and 39%, respectively.

The mean pressure drop within the 2 min after the last inflation of the compliance measurement was 6.5, 7.1, and 7.2 cm H2O for baseline, methacholine, and isoprenaline, respectively. The mean specific pressure drop for these conditions was 0.009, 0.001, and 0.001 cm H2O mm⁻². The differences between the three conditions were not significant.

The collapsibility of the first cycle was 0.6, 0.5, and 0.2 cm H2O below Pcol of the next cycles for baseline, methacholine, and isoprenaline, respectively (all p < 0.001) (Figure 4). Pcol after isoprenaline was 1.5 and 1.6 cm H2O above the values at baseline and with methacholine, respectively (both p < 0.001). Collapsibility at baseline and after methacholine were not significantly different. After maximal contraction, there were five airways that collapsed at pressures of 0 cm H2O or higher.
constriction was methacholine, the less collapsible it was.

The cycles, compliance (sCdyn). (After maximal relaxation, this number increased to nine. Pcol

5 M); Iso

circles denote comparisons between

closed squares

methacholine (10

to 58) %.

Airway Dimensions

The intra- and interobserver variability were well below 10% and 15%, respectively, for each of the airway wall dimensions. There was no systematic relationship between airway size (Pbm) and the intra- or interobserver variability for any variable. The mean coefficients of variation of the individual means for Pbm, WA bm, WA o, WA t, WA rm, WA cart, H epi, and F epi were 4, 6, 8, 10, 33, 12, and 13%, respectively. Characteristics of the airways are shown in Table 3. Highly significant (p < 0.001) linear relations were found between the square root of all airway wall areas and P bm. There was a positive correlation between H epi and P bm (r = 0.43, p = 0.02); in other words, the epithelial layer in large airways was higher compared with small airways. Furthermore, there was a positive correlation between F epi and P bm (r = 0.39, p = 0.04), meaning there was more loss of epithelium in smaller airways. The average H epi was 10.7 ± 6.3 (0.1 to 28.7) μm and F epi was 53 ± 24 (1 to 96) %. The membranous airways disease score was 119 ± 40 (38 to 223).

Lung Function versus Compliance,

Hysteresis, and Collapsibility

Specific hysteresis at baseline correlated to FEV₁/FVC (r = -0.57, p = 0.06) and RV/TLC% pred (r = 0.5, p = 0.05). Hysteresis after methacholine correlated to FEV₁/FVC (r = -0.68, p = 0.02), RV (r = 0.5, p = 0.05), and RV/TLC% pred (r = 0.67, p = 0.007). There was no correlation between hysteresis and parameters of reversibility (ΔFEV₁% pred and ΔFEV₁% ini). Specific compliance and collapsibility did not correlate to the parameters as mentioned previously.

Airway Dimensions versus Compliance,

Hysteresis, and Collapsibility

Specific compliance (sCdyn) at baseline, after methacholine, and after isoprenaline was independent of airway size (p = 0.2). Specific compliance at baseline correlated significantly to the total wall area (WA t) (r = -0.35, p = 0.04) and outer wall area (WA o) (r = -0.39, p = 0.02), not to other airway wall areas. Because the WA t components WA epi and WA bm did not correlate to sCdyn, we conclude that of all factors studied WA o is the most important component for sCdyn at baseline, accounting for 15% of the variability. After methacholine, sCdyn correlated negatively to the amount of smooth muscle (WA m) (r = -0.37, p = 0.05) and to the area of epithelium (WA epi) (r = -0.37, p = 0.05). Because of the strong correla-

of the estimated airway volume. For the relaxing airway after isoprenaline these values were 5.9 (−7 to 46) μl or 31.4 (−14 to 58) %.

Airway Dimensions

The median fluid volume displaced by methacholine-induced constriction was −9.4 (−41 to 10) μl or −37 (−103 to 119) μl or 31.4 (−14 to 58) %.

AFTER Figure 4. Dynamic properties of isolated airway segments at baseline, after methacholine, and after isoprenaline. (A) Specific compliance (sCdyn). (B) Specific hysteresis (sH). (C) Collapsibility Pcol.

The error bars represent the SEM. Closed circles represent the first cycles, closed squares the average of the second, third, and fourth cycle. Arrows denote comparisons between squares. B = baseline; MCh = methacholine (10−4 M); Iso = isoprenaline (10−4 M). *p = 0.03; **p < 0.001.

After maximal relaxation, this number increased to nine. Pcol and sCdyn after methacholine were significantly correlated (r = 0.54, p = 0.001). In other words, the stiffer the airway after methacholine, the less collapsible it was.

The median fluid volume displaced by methacholine-induced constriction was −9.4 (−41 to 10) μl or −37 (−103 to 119) μl or 31.4 (−14 to 58) %.

AFTER Table 3

DIMENSIONS OF ISOLATED AIRWAY SEGMENTS

<table>
<thead>
<tr>
<th>Airway Dimensions</th>
<th>Mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal perimeter, mm</td>
<td>6.1 ± 1.2 (4.1–8.4)</td>
</tr>
<tr>
<td>Airway wall thickness, mm</td>
<td>0.3 ± 0.1 (0.11–0.61)</td>
</tr>
<tr>
<td>Length of airway segment, mm</td>
<td>14.2 ± 4.2 (6.7–21.2)</td>
</tr>
<tr>
<td>Equations of airway dimensions (in mm) as a function of airway size</td>
<td></td>
</tr>
<tr>
<td>Wall area basement membrane, WA bm</td>
<td>( W_{A bm} = 0.19 + 0.071 P_{bm} )</td>
</tr>
<tr>
<td>Outer wall area, WA o</td>
<td>( W_{A o} = -0.15 + 0.206 P_{bm} )</td>
</tr>
<tr>
<td>Total wall area, WA tot</td>
<td>( W_{A tot} = -0.06 + 0.226 P_{bm} )</td>
</tr>
<tr>
<td>Smooth muscle area, WA m</td>
<td>( W_{A m} = 0.04 + 0.034 P_{bm} )</td>
</tr>
<tr>
<td>Cartilage area, WA cart</td>
<td>( W_{A cart} = -0.18 + 0.067 P_{bm} )</td>
</tr>
<tr>
<td>Epithelial area, WA epi</td>
<td>( W_{A epi} = -0.01 + 0.042 P_{bm} )</td>
</tr>
</tbody>
</table>

AFTER
tions of WA_m and WA_epi, no conclusion about which of the two variables was more predictive for sCdyn could be obtained using multiple regression analysis. Specific compliance after isoprenaline did not correlate to any of the airway dimensions. The difference between sCdyn after isoprenaline and methacholine correlated significantly to WA_m (r = 0.45, p = 0.02) but not to WA_epi. The difference between sCdyn at baseline, after methacholine, and isoprenaline did not correlate to any airway wall dimension.

Specific hysteresis (sH) at baseline was positively correlated to WA_t (r = 0.41, p = 0.04) and to WA_m (r = 0.44, p = 0.04), but not to other airway wall dimensions (WA_btm, WA_cart, WA_epi). Because WA_t and WA_m were significantly correlated, it was not possible to differentiate whether WA_t or WA_m was the most predictive dimension for sH in a multiple regression model. Specific hysteresis after isoprenaline correlated to WA_t (r = 0.54, p = 0.006), WA_o (r = 0.52, p = 0.01), and WA_m (r = 0.6, p = 0.004), and to WA_cart (r = 0.42, p = 0.04). Multiple regression analysis showed that WA_m was the only remaining significant predictive dimension (p = 0.01). Specific hysteresis after isoprenaline did not correlate to any of the airway dimensions. The difference between sH1 and sH at baseline, after methacholine, and after isoprenaline did not correlate to any airway wall dimension.

The specific pressure drop after the fourth inflation, indicating hysteresis and leak, was correlated negatively to all airway dimensions for baseline, after methacholine, and after isoprenaline (p < 0.01). Multiple regression analysis showed that at baseline, WA_epi was the only remaining significant predictive dimension for the pressure drop. After methacholine, WA_o was the only significant dimension (p = 0.04) determining the pressure drop. After isoprenaline, multiple regression analysis showed that WA_o and WA_epi were both predictive. Because of the strong correlations between the two, no conclusion about which variable was most predictive could be obtained. Thus, small airways with thinner epithelium and smaller outer airway wall area had a faster pressure drop after the final inflation.

Pc01 of the airways at baseline, after methacholine, and after isoprenaline did not correlate to any of the airway wall dimensions (WA_btm, WA_o, WA_t, WA_m, WA_cart, WA_epi).

DISCUSSION

Chronic inflammation of the airways results in fibrous tissue deposition and thickening of the airway wall. We thought it likely that this remodeling makes the airway less compliant. Our results show that of the airway dimensions studied the bronchial smooth muscle area, not total wall area, is the most important determinant for airway compliance, hysteresis, and collapsibility.

Compliance

A flow compliance is an important predictor of airway diameter and lung compliance (26, 27). We expected that the inner and outer wall area of airways from smokers with varying degrees of airflow obstruction would be important determinants of sCdyn for a number of reasons. The inner and outer wall area correlated positively to inflammatory changes within the airway wall in smokers (1, 11). Furthermore, chronic airway inflammation is related to an increased amount of fibrous tissue within the airway wall (10, 11, 23) which is likely to make the airway stiffer. Though sCdyn correlated to the outer airway wall area at baseline tone, no correlation was present between the outer and inner wall area and sCdyn after maximal relaxation of bronchial smooth muscle tone. A flow maximal contraction of the smooth muscle, sCdyn decreased and smooth muscle area was the most important airway dimension for sCdyn. We believe that the weak correlation between WA_m and sCdyn may well underestimate the importance of smooth muscle for airway compliance. Our measurement of WA_m included not only smooth muscle cells but also connective tissue within the smooth muscle bundles, which increases the variability of the WA_m measurement. Furthermore WA_m is likely to be a poor estimate of the stress that can be generated by the smooth muscle as this is also dependent on the orientation of the muscle bundles in the airway wall, which was not assessed in our study. How can we explain that the inner and outer wall area did not correlate to sCdyn? It could be that the airways we investigated were not thickened by chronic inflammation. We think this is unlikely because the selection criteria for patients and lung tissue were the same as for previous studies (1, 11, 23–25). Furthermore, we investigated small airways, where inflammatory changes are known to be most severe (1). Finally, inflammation scores were within the same range as in a previous study (1) and these scores are known to correlate positively to the inner and outer airway wall area. The lack of correlation between inner and outer wall area and sCdyn could also be explained by assuming that these areas correlate poorly to the presence of structural components important for sCdyn such as collagen.

Specific compliance at baseline was two-thirds of sCdyn of the maximally relaxed airway. The initially high intrinsic contractile state could partly be an artifact related to our tissue preparation. Loss of epithelium was present in many of the airways we studied. Epithelium is a well-known source of bronchodilating substances (28) and its loss might therefore have increased smooth muscle tone. However, sCdyn at baseline did not correlate significantly to the percentage of the basement membrane covered by epithelium. Furthermore, sCdyn at baseline in our study was only slightly higher than other in vivo studies of human airways, taking into account differences in methodology (29–32). If there is a high smooth muscle tone in vivo too, then it is puzzling why none of the patients in our study and only one of 58 patients in a previous study showed reversibility after bronchodilatation (1). How can this discrepancy be explained? In our experimental setup airways were inflated by a mechanical pump. In vivo, airways are dilated by tethering forces of alveolar attachments in relation to breathing cycles. We speculate that in lungs of patients with COPD loss of alveolar attachments results in absence or incomplete dilation of segments or complete membranous airways, even after adequate relaxation of bronchial smooth muscle.

Hysteresis

Smooth muscle tone is known to be an important contributor to hysteresis (33, 34). We found a significant decrease of sH1 with maximal relaxation compared with baseline and with the maximally contracted airways. However, we did not find a difference between sH1 at baseline and after maximal contraction of the smooth muscle. This can be explained by the smaller increase in volume necessary to inflate a contracted airway to the pressure limit of 15 cm H2O. Surprisingly, a substantial portion of the sH1 was not contributed by smooth muscle tone. A flow maximal relaxation, mean sH1 was still 53% of baseline sH1. Hysteresis of lung tissue is related to four different mechanisms: kinetics of cross-bridge attachment-detachment, kinetics related to the surface film, kinetics of fiber–fiber networking within the connective tissue matrix, and the kinetics of recruitment–derecruitment (35). For the sH1 of fluid-filled relaxed airway segments in our study kinetics of cross-bridge at-
tachment-detachment, surface tension, and recruitment do not play an important role. Therefore, it is likely that $s_{f}$ of relaxed airway segments originates within the connective tissue matrix of the airway wall.

At baseline, $s_{f}$ was correlated both to the smooth muscle area and to total airway wall area. After maximal contraction of the smooth muscle, its area was the single most important airway wall dimension related to $s_{f}$. A maximal relaxation, the total wall area was not correlated to $s_{f}$. These findings suggest that the total wall area does not reflect changes in the fibrous tissue composition which determines $s_{f}$. A surprising finding was that specific hysteresis at baseline and after methacholine correlated to in vivo lung function parameters of airflow obstruction. If not a false-positive finding, this might reflect the role of smooth muscle tone in the pathogenesis of airflow obstruction in COPD.

Collapsibility

To our knowledge, this is the first study on the relation between collapsibility and airway dimensions of human small airways. Collapsibility was correlated to smooth muscle tone and not to any of the airway dimensions. Reduction of smooth muscle tone made the airway more compliant and more collapsible. Airways with the biggest change in compliance between the maximally contracted and maximally relaxed state also had the biggest change in collapsibility. Many airways collapsed at negative pressures close to 0 cm H$_{2}$O or higher. In vivo these airways will depend on parenchymal recoil pressure to remain open. The use of bronchodilators in patients might therefore facilitate airway closure during expiration. The importance of smooth muscle tone for the collapsibility of large airways has been reported for different animal species (36–39). We conclude that collapsibility of small airways will to an important degree depend on smooth muscle tone and parenchymal support.

Effects of Volume History

The mechanical characteristics of the first cycle were substantially different from the subsequent cycles. On the first cycle, airways were less compliant, had a greater hysteresis, and were less collapsible compared with subsequent cycles. Similar findings were obtained earlier in bronchi and tracheal strip preparations from dogs (33, 40). In vivo, the effect of volume history on compliance and hysteresis has been described for normal subjects, asthmatics, and patients with COPD (41–44). In the general population and in COPD, patients a deep inhalation results in bronchodi lation (41, 44). In asthmatics deep inhalation can result in bronchoconstriction or bronchodi lation. In COPD, this effect is reduced but still present after inhalation of bronchodilators (44). We found that the difference in $s_{c}$ and $s_{f}$ between the first and the subsequent cycles persisted even after maximal relaxation by isoprenaline. We therefore conclude that the effect of volume history on $s_{c}$ and $s_{f}$ is not only related to smooth muscle tone but also to connective tissue within the airway wall.

Conclusions

In this study smooth muscle area and tone, but not total wall area, were the most important morphological correlates for the dynamic properties of isolated airways from smokers. We showed that maximal bronchodi lation doubled compliance, reduced hysteresis, and increased collapsibility of airway segments independently of airway wall thickness. The volume history of the isolated airway segments had a substantial effect on compliance, hysteresis, and collapsibility even in the absence of smooth muscle tone.

It was striking that isolated airways showed considerable reversibility of bronchoconstriction whereas in vivo patients with COPD have very little reversibility of airflow obstruction after inhalation of bronchodilators (1, 45). We speculate that in patients with COPD loss of alveolar attachments is responsible for absence or incomplete dilatation of segments of membranous airways, even after effective relaxation of bronchial smooth muscle.

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