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Cartilaginous airway wall dimensions and airway resistance in cystic fibrosis lungs

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ABSTRACT: It is not clear how airway pathology relates to the severity of airflow obstruction and increased bronchial responsiveness in cystic fibrosis (CF) patients. The aim of this study was to measure the airway dimensions of CF patients and to estimate the importance of these dimensions to airway resistance using a computational model.

Airway dimensions were measured in lungs obtained from CF patients who had undergone lung transplantation (n=12), lobectomy (n=1), or autopsy (n=4). These dimensions were compared to those of airways from lobectomy specimens from 72 patients with various degrees of chronic obstructive pulmonary disease (COPD). The airway dimensions of the CF and COPD patients were introduced into a computational model to study their effect on airway resistance.

The inner wall and smooth muscle areas of peripheral CF airways were increased 3.3- and 4.3-fold respectively compared to those of COPD airways. The epithelium was 53% greater in height in peripheral CF airways. The sensitivity and maximal plateau resistance of the computed dose/response curves were substantially increased in the CF patients compared to COPD patients.

The changes in airway dimensions of cystic fibrosis patients probably contribute to the severe airflow obstruction, and to increased bronchial responsiveness, in these patients.

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In most cystic fibrosis (CF) patients, chronic airway inflammation leads to progressive airflow obstruction and increased bronchial responsiveness. Lung function abnormalities in asthma and chronic obstructive pulmonary disease (COPD) are related to airway wall thickening, an increase in the amount of bronchial smooth muscle and changes in the interaction between the parenchyma and airways [1, 2]. Whether similar morphological changes play a role in the pathophysiology of airflow obstruction and bronchial responsiveness in CF is unknown. The morphological features of CF lungs obtained at autopsy have been described in a number of studies [3–5]. Extensive inflammation of the bronchial walls and an increased proportion of the lung volume occupied by bronchi was found in CF lungs compared to controls [3, 4]. It is not clear, however, how these pathological findings relate to the severity of airflow obstruction and increased bronchial responsiveness in CF patients.

Theoretically, the following structural abnormalities can contribute to airflow obstruction in CF. First, loss of respiratory epithelium in CF airways has been described by a number of authors but never quantified [6, 7]. The respiratory epithelium modulates smooth muscle tone by production of relaxing factors and by inactivation of bronchoconstricting agents and neurotransmitters [8]. Its loss could, therefore, increase smooth muscle tone and shortening. Secondly, extensive inflammation of the bronchial walls and an increased proportion of the lung volume occupied by bronchi was found in CF lungs compared to controls [3, 4]. From previous studies, it is known that thickening of the wall of small cartilaginous airways is an important determinant of airflow obstruction in patients with COPD [9] and asthma [2, 10]. It is considered likely that cartilaginous airways in CF are thickened, but this has never been quantified. Finally, loss of airway cartilage might contribute to airflow obstruction in CF patients. Airway cartilage is an important structural contributor to the ability of the airway wall to resist deformation during forced expiration. Previous studies in patients with COPD have suggested that cartilage volume is reduced in relation to airway inflammation [11]. This has not been confirmed in other studies [12, 13]. It is not known whether cartilage volume is reduced in CF.

The aim of the present study was to measure cartilaginous airway wall dimensions in CF lungs, and to study the relationship between airway dimensions and airway resistance using a computational model. CF lungs were obtained by means of lung transplantation, lobectomy and

autopsy. Airway wall dimensions and epithelial loss were measured by means of computerized morphometry. The wall dimensions of CF airways were compared to those of patients with COPD with mild-to-moderate airflow obstruction from a previous study [9]. These airway dimensions are well defined since lung specimens can be obtained in high numbers from patients operated on for a peripheral lung tumour. The airway dimensions of the CF and COPD patients were inserted into a computational model to study the dose/response relationship of airway resistance against an increasing dose of a hypothetical bronchoconstricting agent.

Methods

Study population

Lung tissue was obtained from 20 CF patients. In the lung tissue from three patients, no intact airways could be identified; these patients were therefore excluded from further analysis. The lung tissue from the remaining 17 CF patients was obtained by means of lung transplantation (n=12), lobectomy (n=1) and autopsy (n=4). All autopsy lungs came from patients who had died due to respiratory failure. Autopsy was carried out within 48 h after death. The cartilaginous airway dimensions of CF patients were compared to those of 72 patients operated on for a solitary peripheral lung lesion as described in previous studies [9]. Most of these patients were smokers with various degrees of airflow obstruction and are defined as COPD in the present study. The airway dimensions of these COPD patients were used for comparison since they are well defined and show only relatively mild changes in relation to airway inflammation. Epithelial height and the fractional loss of epithelium was measured in a subgroup of 22 of these 72 COPD patients [14]. This protocol was approved by the institutional review board for human studies. The clinical data of the CF and COPD patients are summarized in table 1.

Lung function studies

For CF patients, the most recent lung function tests performed prior to lung transplantation or autopsy were obtained from records held on file. These were carried out in three different lung function laboratories. Dynamic lung volume results could be obtained from all patients. Static lung volumes were available for 11 of the 17 CF patients. Data were expressed as a percentage of the predicted value [15]. The ratio between forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) was expressed as an absolute percentage and as a percentage of the predicted values. For the four CF patients below the age of 18 yrs, the prediction equations of ZAPLETAL *et al*. [16] were used. The lung function of the group of 72 COPD patients was tested within 1 week prior to surgery in a single laboratory as described elsewhere [9].

Table 1. – Study population characteristics and lung function

	CF	COPD
Age	24±9 (7–45)	61±9.5 (37–83)
Male/Female	10/7	54/18
Smoking pack-yrs	0	54.7±34.5 (0.4–180)
Current smokers	0	45
TLC % pred	110±17 (80–152)	109±15 (81–154)
FRC % pred	147±33 (104–197)	123±24 (70–177)
RV % pred	284±54 (199–368)	133±34 (66–219)
FEV_1 % pred	27±16 (36–105)	94±18 (58–135)
FVC % pred	41±17 (14–78)	96±13 (64–134)
FEV_1/FVC % pred	56±19 (36–105)	92±12 (55–114)

Data are presented as absolute values or mean±SD (range), and were determined for 17 (11 for total lung capacity (TLC), functional residual capacity (FRC) and residual volume (RV)) cystic fibrosis and 72 chronic obstructive pulmonary disease (COPD) subjects.

Morphological studies

The central bronchi of the CF transplant recipients' lungs were gently washed to remove sputum, with 0.9% saline delivered through a fine catheter. This lavage was continued until the return fluid was clear. Next, the lung was fully immersed in a large volume of 10% formalin and fixed for ≥24 h. The fixed specimens were serially sliced at 1-cm intervals in a sagittal plane. Intrapulmonary cartilaginous airways cut in cross-section were randomly selected from each specimen for morphometric analysis. Tissue blocks containing cartilaginous airways were decalcified, embedded in paraffin and cut at a thickness of 5 μm. The CF airways were stained with a combined Gomori's trichrome and Gomori's elastin stain. This stain resulted in good colour contrast between airway wall structures and secretions within the lumen. The airways of COPD patients were stained with haematoxylin and eosin and using the Masson trichrome technique.

Measurement of airway dimensions

Sections from cartilaginous airways from CF patients that were transversely cut and did not show bifurcation or disruption of the wall were selected for measurement. The measurements made are shown in figure 1 and include: basement membrane perimeter (PBM) and the area of the lumen including the respiratory epithelium bound by the basement membrane (ABM); the outer muscle perimeter

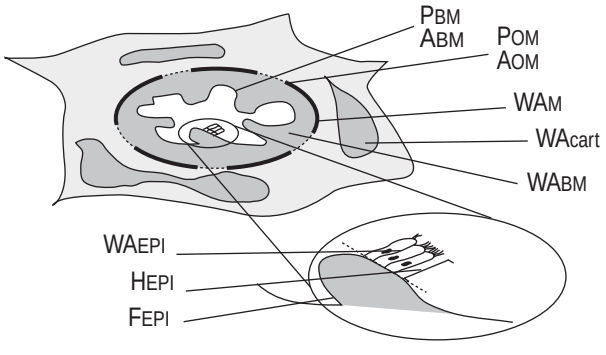

Fig. 1. – Measured airway dimensions. PBM: basement membrane perimeter; ABM: basement membrane area; POM: outer muscle perimeter; AOM: area enclosed by POM; WAM: smooth muscle wall area; WA_{cart}: cartilage wall area; WABM: inner wall area (AOM-ABM); WAEPI: epithelial wall area; HEPI: epithelial height; FEPI: fraction of PBM covered by epithelium.

(POM) and the area enclosed by this perimeter (AOM); the wall area occupied by smooth muscle (WAM); and the wall area occupied by cartilage (WAcart). From these measurements, the inner wall area (WABM=AOM-ABM) was calculated. Nomenclature proposed for the quantification of subdivisions in the bronchial wall was used [17]. Furthermore, the height of the respiratory epithelium (HEPI) and the fraction of the PBM covered by epithelium (FEPI) were measured. HEPI was measured as follows. First, a grid containing parallel sinusoids was superimposed over the computer image of the airway. The probability of the sinusoidal grid line intersecting the basement membrane is random and independent of the orientation of the membrane. Secondly, when respiratory epithelium was present at the point at which the sinusoid crossed the basement membrane, the epithelial height was measured by drawing a straight line perpendicularly from the membrane to the top of the ciliary border. The length of this line was computed automatically. When respiratory epithelium was absent, the height was regarded as zero. Thirdly, HEPI was calculated for each airway section by computing the mean of ≥15 epithelial height measurements around the lumen. Sites at which respiratory epithelium was absent were not included in this mean. FEPI was calculated by dividing the number of intersecting points through the basement membrane covered by respiratory epithelium by the total number of intersecting points, including the intersecting points at sites at which the respiratory epithelium was absent. The wall area covered by epithelium (WAEPI) was calculated by multiplying PBM by HEPI and FEPI. For COPD patients, airway dimensions were measured using the same morphometric approach as described above [9, 14].

Histological staining and image analysis

The wall dimensions of airways from CF patients were measured on Gomori′s trichrome- and elastin-stained sections using an automated image analysis system (KS400; Kontron Elektronic, Eching/Munich, Germany). Airways too large to be recorded in one image were recorded in four separate images that were merged to form a single image. Measurements of airway dimensions were performed by two observers (H. de Bruin and L.P. Koopman). Each of the observers measured a different set of airway dimensions. The intraobserver variability was assessed by remeasurement of 10 randomly selected airways after an interval of 2 months. To evaluate the possible influence of the different methodologies used in this study and the COPD study, the interstudy variability was assessed as follows. Fifteen haematoxylin and eosin-stained airways of COPD patients that had previously been measured by an observer H.A.W.M. Tiddens were selected randomly [9]. Recuts were taken from the tissue blocks of these airways and sections were stained with a Gomori′s trichrome and Gomori′s elastin stain. Next, these sections were measured using the KS400 system by another observer (LK). The interstudy variability, therefore, is the sum of the variability between two observers, two image analysis systems, two different sections and two staining techniques.

Statistical analysis

The intraobserver and interstudy variability were calculated by expressing the difference between the first and second measurements as a percentage of the mean of both observations [18]. This percentage was plotted against PBM to detect systematic errors dependent on airway size. Furthermore, the mean and SEM of the compared measurements were calculated. Finally, the interclass correlation coefficients for the repeated measures were calculated to assess agreement.

Repeated measurement analysis of variance (RMANOVA), which allows for differences between and within patients, was used to assess the relationships between airway wall dimensions (WABM, WAM, WAcart, HEPI, FEPI) and airway size (PBM) [19, 20]. RMANOVA analyses the patients as a continuum and thus avoids the bias that would result from forming subgroups of patients. The previous work of TIDDENS *et al*. [9], KUWANO *et al*. [10] and BOSKEN *et al*. [21] found linear relationships between the square root of airway wall areas and airway size. Square root transformation of the present data (WABM, WAM, WAcart) again resulted in normal distributions of data around the linear regression lines. In the analysis, the airway sizes were centred by subtracting the mean PBM of 14 mm from all values of PBM. The intercept of the individual regression lines for individual patients of a particular airway wall dimension, therefore, denotes its level at a PBM of 14 mm. The mean slopes and intercepts of the CF patients were compared with those of the COPD patients. The level of significance was set at $p=0.05$ (two sided). Data are expressed as mean±SD and range, unless indicated otherwise.

Airway dimensions and computational model for airway resistance

To predict the effect of airway dimensions on airway resistance, a computational model, as described by WIGGS *et al*. [22] and modified by LAMBERT *et al*. [23], was used. Briefly, the geometry of the bronchial tree in the model is a dichotomously branching network with 16 generations. The model is designed to examine the relative contributions of airway wall dimensions to airway responsiveness. The dose/response relationship of airway resistance against an increasing dose of a hypothetical bronchoconstricting agent that causes smooth muscle shortening was calculated. In the model, the airway smooth muscle in each generation of the tracheobronchial tree contracts in response to the agonist and shortens until the stress generated by the muscle is maximal. The plateau of the dose/response curve is achieved when the muscle stress in all airway generations is maximal. The model takes account of both the geometry and the mechanics of the airway and parenchymal tissue.

The computational model used the following airway wall dimensions. For COPD patients, airway dimensions ($\sqrt{}$WABM and $\sqrt{}$WAM *versus* PBM) were derived from a single database containing patients with various degrees of airflow obstruction (St Paul′s Hospital, Pulmonary Research Laboratory, Vancouver, Canada). For membranous airways (PBM 2–4.7 mm), equations were derived from the study of KUWANO *et al*. [10]. For cartilaginous airways

(PBM 4.7–58 mm), equations were derived from the study of TIDDENS *et al*. [9]. For CF patients, the same equations for membranous airways as for the COPD patients were used [10]. For cartilaginous airways of CF patients, the equations ($\sqrt{}$WABM, $\sqrt{}$WAM and $\sqrt{}$WAEPI *versus* PBM) derived from the present study were used as shown in figure 2. Differences in the computed dose/response curves of CF and COPD airways are therefore the result of differences in cartilaginous airway dimensions only.

In the model, it was assumed that the WAM correlates with force in a linear fashion. From the simulated dose/response curves, baseline resistance, the dose of a hypothetical agonist that caused a 10-fold increase in resistance (PD10), and the maximal plateau resistance achieved were calculated.

Results

Lung function

The lung function characteristics of the CF and COPD patients are shown in table 1. All CF patients had severe airflow obstruction (mean±SD (range) FEV1/FVC 33±3 (29–38)%). Of the group of 72 COPD patients, 24 had a maximum expiratory airflow within the normal range (FEV1/FVC >75%), 21 showed a mild reduction (FEV1/FVC 65–75%) and 27 a severe reduction (FEV1/FVC <65%).

Morphological studies

In the group of CF patients, morphometric measurements were made on 88 cartilaginous airways. The mean number of airways per patient was 4.8 (1–11). The range of the PBM of the cartilaginous airways was 5.9–38 mm, corresponding to an airway diameter of 1.9–12 mm or 12th generation to mainstem bronchus [24]. In the group of 72 COPD patients, measurements were made on 341 cartilaginous airways, a mean of 4.7 (3–7) airways per patient. The range of the PBM was 5–34 mm, corresponding to a diameter of 1.5–10.9 mm [9]. The intraobserver variability was -1.5±0.85% for PBM, 2.6±2.17% for WABM, 7.8±11.2% for WAM, -4.8±3.31% for WAcart and 1±4.03% for HEPI. The interclass correlation coefficients for these airway dimensions were all >0.9. The interstudy variability was 4.6±2.6% for PBM, -5.8±7.5% for WABM, -3.5±11.8% for WAM and -6±20.7% for WAcart. The interclass correlation coefficients were 0.96, 0.92, 0.84 and 0.86, respectively. There was no systematic relationship between airway size (PBM) and the intraobserver or interstudy differences for any variable. For CF patients, highly significant ($p<0.001$) linear relations were found between airway wall dimensions ($\sqrt{}$WABM, $\sqrt{}$WAM and $\sqrt{}$WAcart) and airway size (PBM) (fig. 2) and between HEPI and PBM. For COPD patients, the highly significant ($p<0.001$) linear relations between $\sqrt{}$WABM, $\sqrt{}$WAM, $\sqrt{}$WAcart and HEPI and PBM are described elsewhere [9, 14].

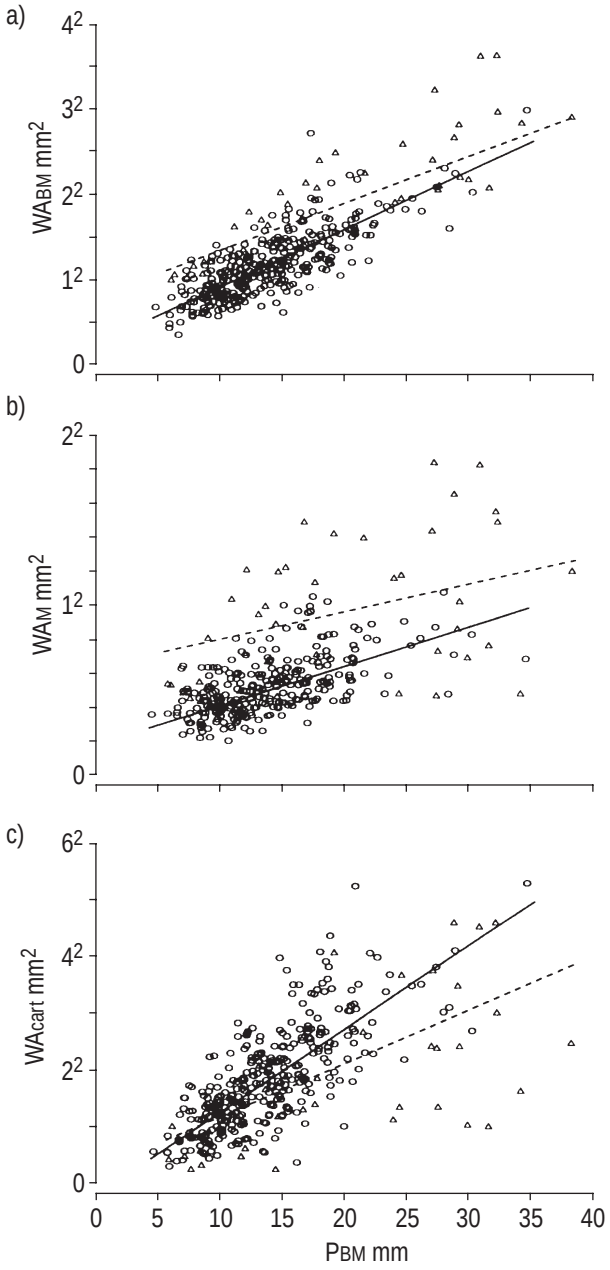

Fig. 2. – Morphometric airway dimensions *versus* airway size: a) inner wall area without epithelium (WABM); b) wall area occupied by smooth muscle (WAM); and c) wall area occupied by cartilage (WAcart). The data comprise 88 points from 17 cystic fibrosis (CF, △) and 341 points from 72 chronic obstructive pulmonary disease (COPD, ○) patients. Due to overlap of data points, some symbols appear solid. The regression lines represent airway dimension *versus* basement membrane perimeter (PBM) for CF (- - - -) and for COPD (——) patients. The regression equations are as follows: a) CF: $\sqrt{}$WABM=0.778+0.058 PBM, COPD: $\sqrt{}$WABM=0.236+0.071 PBM; b) CF: $\sqrt{}$WAM=0.62+0.017 PBM, COPD: $\sqrt{}$WAM=0.21+0.023 PBM; and c) CF: $\sqrt{}$WAcart=0.246+0.096 PBM, COPD: $\sqrt{}$WAcart=-0.168+0.147 PBM.

Airway dimensions

Figure 2 shows cartilaginous airway dimensions ($\sqrt{}$WABM, $\sqrt{}$WAM, $\sqrt{}$WAcart) *versus* airway size (PBM) for CF and COPD patients. The regression lines $\sqrt{}$WABM and $\sqrt{}$WASM *versus* PBM were at a higher level in CF

Table 2. – Ratios between airway dimensions in the PBM range 5–35 mm in cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD)

	Airway dimension: ratio CF/COPD			
PBM	WABM	WAM	WAcart	HEPI
5	3.3	4.7	–	1.6
10	2.1	3.2	0.9	1.4
15	1.7	2.5	0.7	1.3
20	1.4	2.1	0.6	1.2
25	1.2	1.8	0.6	1.2
30	1.1	1.6	0.5	1.1
35	1.1	1.4	0.5	1.1

PBM: basement membrane perimeter; WABM: inner wall area without epithelium; WAM: smooth muscle area; WAcart: cartilage area; HEPI: epithelial height.

patients than COPD patients ($p<0.001$ and $p<0.01$). $\sqrt{}$WAcart *versus* PBM showed a less significant correlation in CF patients than COPD ($p<0.01$). Table 2 shows the ratios between the airway dimensions in CF and COPD airways in the PBM range 5–35 mm. HEPI increased linearly with airway size in CF patients according to the equation HEPI=39.5 μm+1.18×10^{-3}PBM (μm). The HEPI of cartilaginous CF airways was on average 18.2 μm greater than for COPD airways ($p<0.01$). FEPI was 0.7 for CF and did not correlate with airway size.

For airway dimensions of CF patients, airways obtained from transplantation, lobectomy and autopsy were used. To assess any bias because of this, the analysis was repeated without the 16 airways obtained from autopsy and lobectomy. This had no significant influence on the equations for airway dimensions ($\sqrt{}$WABM, $\sqrt{}$WAM, $\sqrt{}$WAcart, and HEPI) *versus* airway size (PBM). The fraction of the PBM covered by epithelium increased from 70 to 77%. The differences between the airway dimensions of CF and COPD patients remained highly significant.

Airway dimensions and airway resistance

Figure 3 shows the dose/response relationship of airway resistance against the logdose of a hypothetical bronchoconstricting agent for CF and COPD airways. The curves are shown for CF and COPD without epithelium, taking epithelial loss into account, and with intact epithelium. Since it was found that the area of the smooth muscle was increased in CF compared to COPD, this force generated by the smooth muscle would be increased for CF in the model. Because it is not known whether airway smooth muscle force is increased in CF patients, the dose/response relationship for CF was studied, first using the WAM equation of COPD. This simulation represents the effect of WABM and WAEPI on airway resistance without increased smooth muscle force. The simulations using the WAM equation of CF represent the effect of WAM and WAEPI on airway resistance with increased smooth muscle force.

The baseline resistance for CF was 32–219% higher than in COPD for the different simulations. The PD10 for CF was 0.3–0.6 logdose below that of COPD patients. The maximal resistance was 215 $cmH_2O\cdot L^{-1}\cdot s$ higher in CF than in COPD when simulated without WAEPI and with the

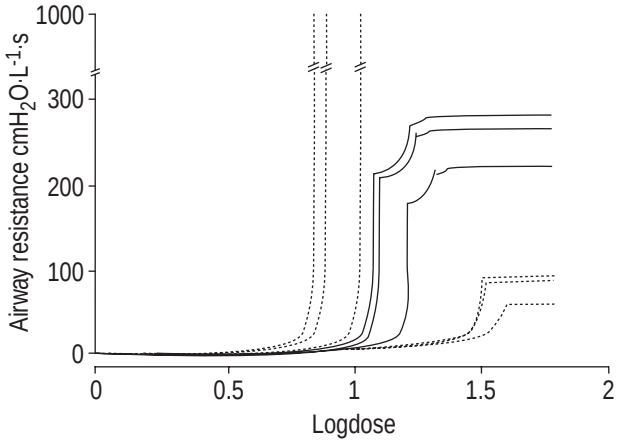

Fig. 3. – Simulated resistance/dose curves to study the effect of airway dimensions on airway resistance in chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF). The lines represent the dose/response relationship of airway resistance against an increasing dose of a hypothetical bronchoconstricting agent. Computations were performed using the airway dimensions of the following three groups of patients: COPD (·····); CF with smooth muscle dimensions of COPD patients (——); and CF with smooth muscle (- - - -). For each of the three groups, the following simulations were carried out: 1: without epithelium; 2: with epithelium but taking epithelial loss into account; and 3: with intact epithelium. The shoulders in the CF with COPD smooth muscle lines show the moment when the smooth muscle tension equals the load in a given generation of the bronchial tree. When this is the case in all generations, the plateau is obtained.

WAM of COPD for both CF and COPD. When the WAM equation was changed from COPD to CF in CF, and therefore the force of the smooth muscle increased, a maximal plateau resistance was not reached below a resistance of 1,000 $cmH_2O\cdot L^{-1}\cdot s$, at which point the calculation was stopped. The maximal resistance in COPD increased from 49 to 83 $cmH_2O\cdot L^{-1}\cdot s$ when the epithelium was added to the model.

Discussion

To the authors' knowledge, this is the first study in which the dimensions of a large number of airways have been measured in a group of CF patients suffering from end-stage lung disease. The airway dimensions of CF patients were compared to those of COPD patients with varying severity of airflow obstruction. Furthermore, the effects of these dimensions on airway resistance and responsiveness were studied in a computational model. The results show that CF patients exhibit a significant increase in airway wall area and smooth muscle area and a decrease in cartilage area compared to COPD patients. In addition, a high percentage of epithelial loss was found in these patients. The changes in airway dimensions in CF patients are likely to contribute to the severe airflow obstruction, increased bronchial responsiveness and reversibility described in these patients.

Airway dimensions

A substantial increase in the inner wall area and smooth muscle area was found in CF patients compared to COPD patients. This increase was larger in small cartilaginous

airways than in central airways (table 2), and would be even more impressive if compared to the airways of healthy controls for two reasons. First, CF airways were compared to those of COPD patients in which the inner airway wall is also thickened [9]. Secondly, it is likely that there was a selection bias towards analyses of the better airways present in the CF lungs. Many airways had to be excluded from the morphometric analysis as they were so heavily inflamed that histological structures could not be identified adequately. The WABM of airways with a PBM of 10 mm of CF patients was increased in comparison to COPD by 210%, which is similar to the WABM in patients who died from asthma as described by KUWANO *et al*. [10]. The WAM of airways with a PBM of 10 mm of CF patients was increased by 320% compared to COPD, and is even 33% higher than that found in patients who died from asthma [10].

Almost one-third of the basement membrane in central and peripheral CF airways was not covered by epithelium compared to 10% in COPD [14]. Loss of respiratory epithelium in CF airways has been described by a number of authors but never quantified [6, 7]. This great loss of epithelium might be related to the washing procedure of the large airways after pneumonectomy. However, this is unlikely since epithelial loss in peripheral cartilaginous airways was as great as that in central airways. Furthermore, it could be the result of necrosis of tissue before fixation. However, even when airways obtained from autopsy specimen were excluded, the fractional loss in CF remained high. Finally, it might be related to increased fragility of the respiratory epithelium in CF patients. In asthma, increased shedding is thought to reflect increased fragility of the respiratory epithelium [25]. Normal respiratory epithelium forms a protective barrier against colonization by bacteria. Its loss in airways of CF patients has been associated with the presence of *Pseudomonas aeruginosa* [26]. Airway epithelial injury and repair favour bacterial adherence and may thus contribute to the airway infection in CF [27]. The respiratory epithelium in airways of CF patients was substantially higher than that of airways from COPD patients [14]. The reasons for the increased height of respiratory epithelial cells in CF are unknown.

A number of authors have suggested a correlation between airway inflammation and the amount of cartilage in COPD patients [11]. Others did not find such a correlation [12, 13]. For CF patients, the area of cartilage in the central airways was reduced compared to COPD. This might reflect loss of cartilaginous tissue due to chronic airway inflammation. The reduced amount of airway cartilage in CF might be due partly to an artefact caused by more airways with bronchiectatic changes having been measured in CF patients compared to COPD. It is likely that the airway size of such airways would be overestimated, and, therefore, the amount of airway cartilage relative to airway size underestimated.

Airway dimensions, airflow obstruction and bronchial responsiveness

With increasing age, severe airflow obstruction develops in most CF patients. In addition, increased bronchial responsiveness to bronchoconstricting agents and bronchodilators develops in up to half of CF patients [28–35]. The pathophysiology of these lung function abnormalities is incompletely understood.

How thickening of the inner wall, smooth muscle and epithelial area can contribute to airflow limitation and bronchial hyperresponsiveness in CF patients was studied through the use of a computational model. Thickening of the inner wall area increased the computed baseline resistance by up to 66% in CF patients when compared to COPD and caused a leftward shift (as assessed by PD_{10}) in the dose/response curve. When the epithelial area was added to the inner wall area, the dose/response curve was shifted further to the left. This was true for both CF and COPD airways. These results show that a leftward shift in the dose/response curve can be caused by a thickened inner wall and that the epithelial area contributes substantially to bronchial responsiveness in CF and COPD. This leftward shift would be reduced by loss of epithelium. The computed resistance plateau height increased with increasing inner wall thickness. When the smooth muscle force was increased in the model by inserting the smooth area found in CF patients, the calculated plateau resistance became unmeasurably high. Conclusions drawn from model studies must always be tempered with the knowledge that they are not reality. However, the model used here has been shown to give results that are in good qualitative accord with those of *in vivo* experiments [23, 36]. Thus the authors are confident that, although the absolute values computed with the model are not expected to match experimental values, trends in computed values should mirror reality.

It is unknown whether it is justified to assume that the WAM correlates with force in a linear fashion. The increase in WAM found in CF airways in the present study might be the result of an increase in the smooth muscle matrix and not of smooth muscle cells [37]. Whether smooth muscle is able to shorten *in vivo* depends not only on smooth muscle force but also on the load on the shortening smooth muscle. On the one hand the load could be increased due to thickening and stiffening of the heavily inflamed inner airway wall area [38, 39]. On the other hand, it could be reduced due to uncoupling of the smooth muscle from the tethering forces of the parenchyma [1]. Loss of parenchymal attachments would reduce the load for the contracting smooth muscle even further. Severe parenchymal destruction was observed in the lung tissue of CF patients, but neither systematically quantified nor taken into account in the computational model.

In vivo measurements of bronchial responsiveness in CF patients demonstrate that smooth muscle shortening occurs, since a large percentage of CF patients show an increased response to bronchodilators [30, 31, 34, 40]. This implies that under baseline conditions the smooth muscle is shortened to some extent. Furthermore, inhalation of directly acting bronchoconstrictors results in increased airway resistance in many patients [28, 29, 32, 33]. The model predicts a reduced provocative dose of methacholine causing a 20% fall in FEV_1 (PD_{20}) in CF patients compared to COPD. The PD_{20} in CF is probably lower than that of the responsive COPD patients in the present study [9, 33]. When CF patients are compared with asthma patients, this PD_{20} is usually higher [29, 33]. Whether the plateau resistance in CF patients is increased

as predicted by the computational model has not been investigated to the authors' knowledge.

The importance of epithelial loss, as found in CF airways for airflow obstruction and bronchial hyperresponsiveness, can only be speculated upon. An important function of the respiratory epithelium is modulation of smooth muscle tone by production of relaxing factors and by inactivation of bronchoconstricting mediators and neurotransmitters [8]. The extensive loss of respiratory epithelium found in CF in the present study could, therefore, increase smooth muscle tone and thus increase airway obstruction and enhance bronchial responsiveness to histamine and methacholine [41]. In asthma, it has been shown that the greater the loss of respiratory epithelium in biopsy specimens the greater the degree of airway responsiveness [42]. Loss of cartilage is likely to make airways more collapsible and might therefore contribute to airflow limitation in CF. It might also contribute to uncoupling of smooth muscle from its surrounding tissue and thus enable increased shortening for a given amount of force generation.

Altered airway dimensions and therapy

Thickening of the inner airway wall in CF airways increases airway resistance and bronchial responsiveness in the computational model. Bronchodilation of thickened airways can substantially reduce airway resistance [2], and thus could be beneficial for CF patients. Bronchodilators are used by many CF patients and have been shown to improve lung function and reduce hospitalization compared to placebo [43].

Airway wall thickness is positively correlated to airway inflammation and airflow obstruction in COPD [9]. It is likely that a similar correlation is present for airways of CF patients. The authors speculate that the positive effects on lung function parameters of antibiotic or anti-inflammatory treatment [44–46] are related to a reduction of the inflammatory changes within the airway wall and thus of airway wall thickening. In addition, antibiotic and anti-inflammatory treatment is likely to reduce ongoing damage to the respiratory epithelium and move the balance towards repair of the epithelial layer and restitution of its functions.

Conclusion

In cystic fibrosis patients with end-stage lung disease substantial thickening of the inner wall area and smooth muscle area was found in comparison to patients with chronic obstructive pulmonary disease. In addition, a reduction in the cartilage area was observed in central airways. Finally, an increased height of the epithelial layer and a high percentage of epithelial loss was found. These changes in airway dimensions are likely to contribute to the severe airflow obstruction, and to the increased bronchial responsiveness to bronchoconstricting and bronchodilating agents, described in cystic fibrosis patients.

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