Chapter 3

Advances in airway and parenchymal physiology and pathobiology

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Complex changes in the structure and function of airways and lung parenchyma occur in different respiratory diseases. Their interactions have profound and sometimes unpredictable effects on the pulmonary function tests used in clinical practice. https://bit.ly/ERSM107

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This chapter reviews the multiscale structure–function relationships of the lung parenchyma, from fibres through surface tension to alveolar septal network, with an emphasis on abnormalities such as emphysema, pulmonary fibrosis, pneumonia and lung metastases, and with consideration of implications for their clinical assessment. Structural abnormalities of the airway wall observed in obstructive disease are described, as are the implications for function at a local tissue level and how these may manifest *in vivo* when considering the heterogeneity of structure–function. We also look at length adaptation, a major property of airway smooth muscle: changes in cell length initially disassemble the contractile apparatus, resulting in a reduction of contractility, which is followed by reassembly of the contractile apparatus and recovery of contractility. This confers on the muscle the ability to modulate airway calibre in response to deep inspirations. Finally, the impact of structure–function characteristics and airway–parenchymal interdependence on standard lung function tests is examined, and we highlight how interferences between various mechanisms may affect overall lung function differently, depending on the underlying disease and severity.

Introduction

The human lung is a complex organ, the function of which is determined by the interactions between structures with different morphological and mechanical properties. A major difference between the lung and other internal organs is that the lung is continuously submitted to external forces throughout life, and dysfunction may occur whenever pathological changes alter the structure or the mechanical behaviour of the airways, lung parenchyma, or both.

This chapter will first describe the structural and functional abnormalities of lung parenchyma in the healthy lung, and in several main parenchymal diseases, such as emphysema, pulmonary

fibrosis (PF) and lung cancer. Next, we will discuss the structural abnormalities of the airway wall in obstructive disease, and the implications for function at a local tissue level in the presence of heterogeneity. We then provide an overview of length adaptation of airway smooth muscle (ASM), with emphasis on how changes in cell length regulate the contractile apparatus in response to deep inspiration (DI). The last section of the chapter examines the impact of structure–function characteristics and airway–parenchymal interdependence on standard lung function tests, highlighting how different mechanisms interact to influence overall lung function, depending on the severity of underlying disease. The way in which disease impacts clinical lung function testing will be illustrated throughout the chapter.

Lung parenchymal structure, mechanics and functional abnormalities

Alveolar structure

The lung parenchyma, by definition, is the structure beyond the respiratory bronchioles, which includes the alveolar ducts, with occasional branching, and the alveoli, where gas exchange occurs (figure 1). This complex structure has been optimised for efficient gas exchange *via* passive diffusion, which requires a large surface area and a small distance between air in the alveoli and capillary blood. The demands of gas exchange impose severe constraints on alveolar structure, including alveolar size and septal wall thickness [1, 2]. For example, the large surface area (~70 m^2) requirement is satisfied by packing ~480 million alveoli into the volume of the lung, with diameters at total lung capacity (TLC) reaching 200 µm in the adult human lung; in

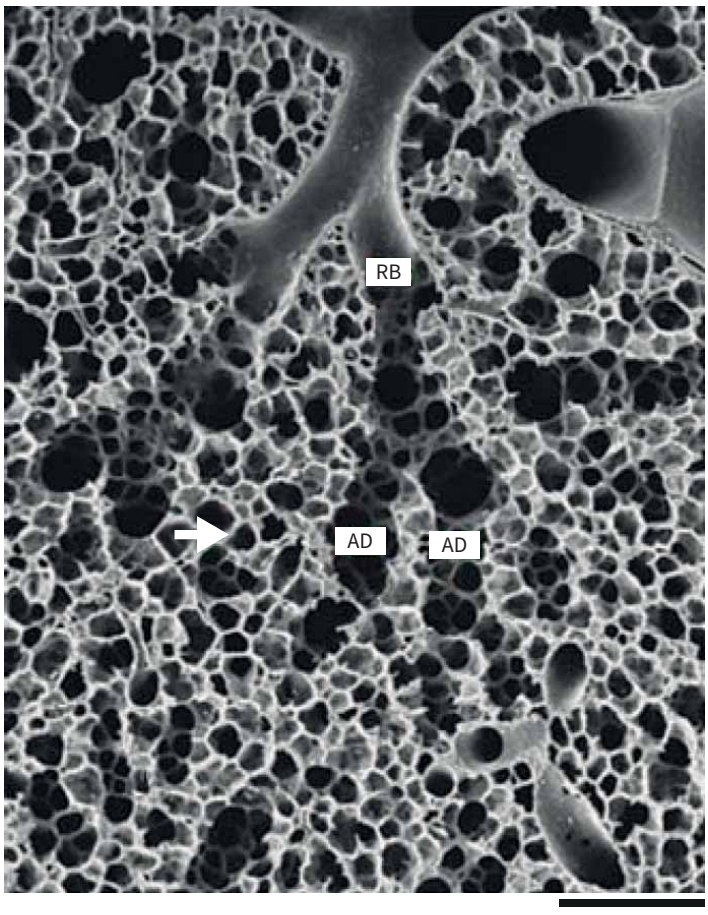

FIGURE 1 Image of the lung parenchyma showing a respiratory bronchiole (RB) and two alveolar ducts (AD). The arrow points to a single alveolus. Scale bar: 500 µm. Reproduced and modified from [1] with permission.

contrast, the short diffusion distance (~1 µm) means that the septal walls separating neighbouring alveoli only need a thickness of <8 µm [3]. This complex interconnected network of thin-walled tubes and spherical gas exchange units displays a fractal organisation [2]. The evolution of the lung tissue has not only fine-tuned parenchymal structure for gas exchange but has also resulted in proper composition of its extracellular matrix (ECM), including elastin and collagen, with mechanical properties that allow easy stretching and force transmission during breathing [4].

Taken together, the healthy lung parenchyma is a well-designed structure suitable to serve an organism's needs both at rest and at maximum effort [1].

Multiscale mechanics

Lung tissue mechanical function derives from ECM and alveolar structure, the mechanical properties of the constituents and their interactions [5]. The primary load-bearing structural protein of the alveolar ECM is thought to be the fibril-forming collagens, including types I and III. The very stiff, rod-like collagen molecules [6] are arranged into hierarchical fibrils and fibres that are stabilised by cross-links [7]. These fibres form: the axial fibre system running from the airways down to the alveolar ducts; the peripheral fibres emanating from the visceral pleura; and the network of fibres in the septal walls linking the axial and peripheral networks [7]. During development [8, 9] and in diseases such as COPD [10] and PF [11], the collagen amount and type can vary in lung tissue. As a result, collagen fibres have been associated with the structural stability and biomechanical properties of the parenchyma. Collagen fibres are also wavy at low and intermediate lung volumes [12]. As the lung inflates, fibres become straighter and, as lung volume approaches TLC, the waves gradually disappear. This recruitment process stiffens the septal walls [13], resulting in stiffer parenchyma, and leading to the saturation-like plateau of the pressure–volume (P–V) curve of the lung [14, 15].

During normal breathing, the elastic fibres provide the majority of recoil pressure of the lung parenchyma [16]. These fibres contain elastin, a soft and resilient biopolymer, surrounded by microfibrils. The stiffness of elastic fibres is at least 100 times lower than the stiffness of fully straightened collagen fibres [6]. The easy stretchability of elastic fibres derives from the entropic nature of the extension of the hydrophobic molecule, coupled with dense cross-linking, which together produce a perfect linear stress–strain curve, with near zero hysteresis up to a strain of ⩾150% [6]. These fibres are also mechanically associated with collagen fibres [17], perhaps through microfibrils or proteoglycans [18–20]. The fibres form a fully connected network in the parenchyma [12], meaning they play a major role in lung elasticity at low-to-medium lung volumes [16], and are primarily responsible for lung compliance during breathing. Due to their superb mechanical characteristics, it has been suggested that elastic fibres behave mechanically as an ideal linear spring that does not dissipate energy during cyclic stretching [6]. Hence, elastic fibres are ideally suited to supporting breathing throughout life.

Several additional factors contribute to parenchymal mechanics. The most important is the pre-existing stress, or prestress, in the septal walls due to transpulmonary pressure [21]. The pleura transmits the mechanical stresses of transpulmonary pressure to the subpleural alveolar septal walls, which in turn transmit these stresses to the neighbouring alveoli, and their next neighbours, and so on, forming a complex interdependent prestressed network. The actual stress transmission occurs through the fibre network [22]. The total stress in alveolar tissue therefore arises from the dynamic stresses caused by breathing, superimposed onto the static prestress. Consequently, all adherent cells, such as epithelial, endothelial, and fibroblast cells, feel and respond to these stresses *via* mechanotransduction, affecting many cellular functions [23, 24].

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An important function of the prestressed alveolar network is that it structurally supports small airways, preventing them from collapse in the normal lung.

In addition to prestress and ECM, lung mechanics is also influenced by the surface tension of the air–liquid interface lining the alveolar surface, especially at low lung volumes [25–28]. Because pulmonary surfactant lowers surface tension as lung volume decreases, alveoli remain stable at end-expiration [28].

Other constituents of the parenchyma that contribute to a lesser extent to lung mechanics include the compressibility of proteoglycans [29] and the elastic properties of adherent cells [30–32].

The effects of disease on alveolar structure and function

How do diseases affect the ECM, the septal walls and their network organisation? Emphysema, one of the major subtypes of COPD, is known to attack and remodel both the elastic and collagen fibres [33–35], which, due to prestress (particularly in the apex of the lung), become prone to rupture [36]. Once fibres in the wall fail, ECM fragments are exposed that can drive further progression [37]. At some point, the wall itself ruptures, redistributing the mechanical load it carried among neighbouring septal walls, and increasing the likelihood that these walls will rupture [38]. This process leads to airspace enlargement, a characteristic feature of emphysema, locally softening the tissue. Furthermore, residual inflammation in the enzymatically weakened septal walls can undergo cyclic stretch-induced fatigue, leading to rupture and causing continued airspace enlargement and functional decline [39], potentially even in the absence of stimulus. In PF, activated myofibroblasts remodel the septal walls [40] through mechanotransduction-driven scar formation [41], resulting in uncontrolled collagen deposition [42] and cross-linking [43]. Subsequent stiffening of the alveolar tissue further activates myofibroblasts in a positive feedback loop [44]. Alveoli affected by pneumonia are usually oedematous, which hinders normal surfactant function [45]. However, little is known about how ECM remodelling influences alveolar mechanics in lung cancer.

In a recent study, Banerji *et al.* [46] introduced novel technology: the crystal ribcage. This technology makes it possible to perform multiscale imaging and probing of the mechanics of single alveoli in the mouse lung as a function of heterogeneous remodelling of the ECM and septal walls in several diseases, including lung metastasis (nodular *versus* infiltrative tumour growth), emphysema and fibrosis. In the study, nodular and infiltrative tumours, also known as co-optive tumours, were created in the lung through intravenous injection of murine breast cancer cell lines. Pneumonia was induced *via* bacterial infection with *Streptococcus pneumoniae* serotype 3, and fibrosis was modelled using bleomycin treatment (figure 2). In the nodular phenotype, tumours were observed to “push” into the lung tissue and bulge out of the pleural surface, whereas infiltrative tumours replaced existing lung tissue and were superficially located on the pleural surface. While this differential growth pattern has been reported previously in brain tumours [47], there is poor understanding of its presence in the context of the lung and how it may affect the surrounding alveoli. Compared with the healthy lung, in pneumonia, alveoli were filled with neutrophils, whereas in fibrosis, injured regions demonstrated thickened septal walls with gross heterogeneity.

Banerji *et al.* [46] achieved mechanical probing of individual alveoli by assessing the pressure–diameter (*P*–D) curves, which revealed a large inter-alveolar heterogeneity in the healthy lung, as well as distinct functional impairments specific to each disease (figure 2b). For example, nodular tumours demonstrated how growth-induced forces, one of the physical hallmarks of cancer [48, 49], affect neighbouring tissue. Alveoli that contained nodular tumours (100–

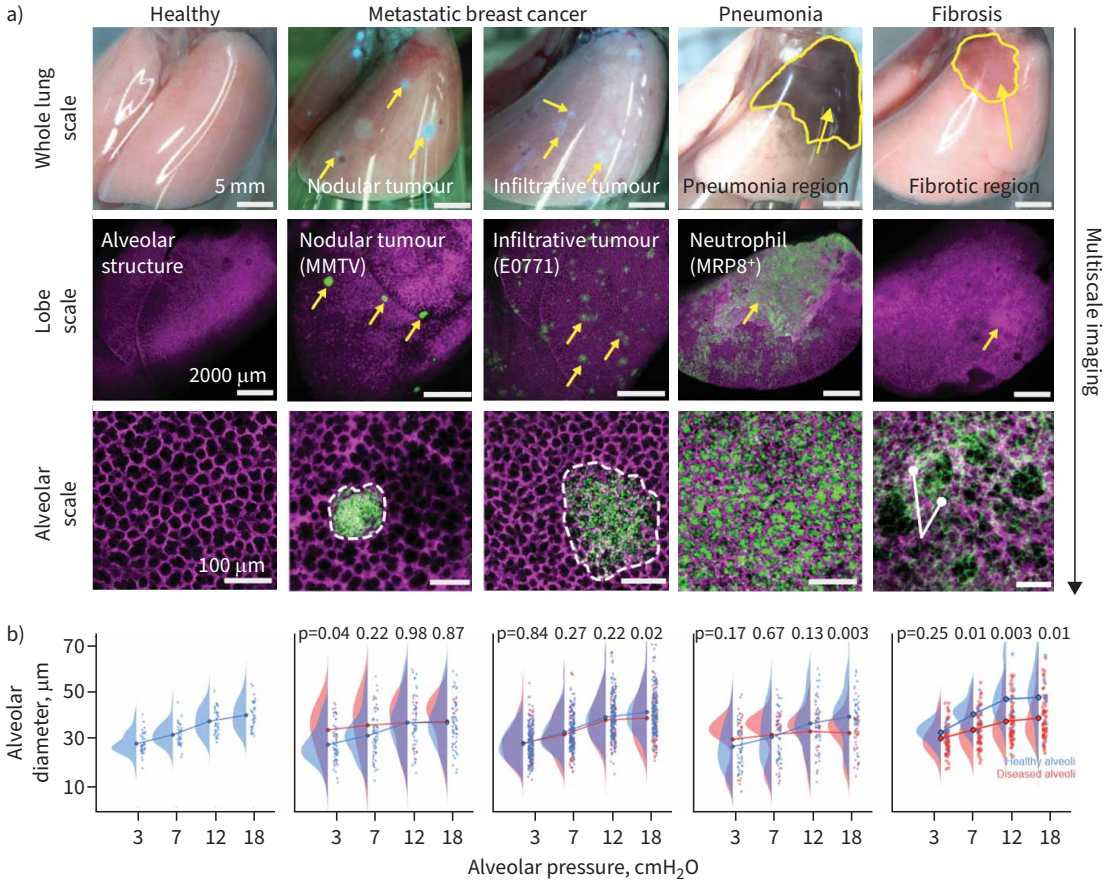

FIGURE 2 a) Multiscale imaging of the lung, from whole organ down to alveoli, in health and disease, and b) the corresponding alveolar pressure–diameter values measured *via* the crystal ribcage platform, with healthy alveoli indicated in blue and diseased alveoli indicated in red. MRP8: reporter for neutrophils. Reproduced and modified from [46] with permission.

200 µm in diameter) were unable to inflate, rendering them non-functional for gas exchange. These alveoli remained maximally dilated across the quasi-static alveolar pressure range, indicating that cancer cells occupied the entire airspace and forced alveoli to remain fully distended. These functional impairments extended to one or two layers of the adjacent alveoli in the peritumoral region. Larger nodules (1–2 mm in diameter) caused alveoli to become stretched and compacted up to 200 µm from the tumour boundary, while maintaining larger diameters at greater distances. In sharp contrast, infiltrative tumours did not impair the mechanical function of alveoli that were inside or adjacent to the tumour. These alveoli behaved similarly to the healthy ones, suggesting that this type of tumour preserved existing tissue architecture, and did not compromise mechanical function. Interestingly, when the elasticity of the lung and tumour areas were mapped with a multiscale mathematical model, both the tumour and nearby normal lung tissue showed substantial strain stiffening, a sign of collagen recruitment, with the tumour regions stiffening at a higher rate [50].

In the pneumonia model, infected alveoli exhibited functional deficits over the range of quasi-static alveolar pressures (3–18 cmH_2O). Alveolar diameters remained largely constant, resembling healthy alveoli at 7 cmH_2O. This loss of expansion and contraction capacity resulted in a very low compliance that may be attributed to neutrophil infiltration, pulmonary oedema, likely caused by surfactant inactivation [45] and compromised vascular integrity [51], allowing

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fluid influx into the airspaces. Finally, in the fibrosis model, the injured alveoli demonstrated a heterogeneous structure with thickened septal walls. The *P*–D curves displayed reduced diameters at higher alveolar pressures compared with normal alveoli, consistent with a stiffening phenotype and lower compliance, as reported in human PF [52].

These findings underscore the structural and functional mechanical alterations in individual alveoli across different pulmonary diseases, highlighting the distinct mechanisms of ECM and septal wall remodelling and dysfunction.

Assessment of parenchymal function

The clinically measurable functional index of the parenchyma is lung compliance, the slope of the lung's *P*–*V* curve, which is an emergent property arising from the mechanical properties of the ECM's fibres, their structural arrangement, the surfactant system and their multiscale interactions. The *P*–*V* curve of a single alveolus is determined by elastin stiffness and collagen waviness in the septal wall and the surface tension along the wall [14]. In health, the *P*–*V* curve of the lung is essentially the sum of the individual alveolar *P*–*V* curves, corresponding to the gravitational position of each alveolus [53]. Lung compliance is therefore sensitive to the mechanisms that govern the *P*–*V* curve of individual alveoli.

In parenchymal diseases such as emphysema, PF, pneumonia and lung cancer, the characteristics of the single alveolar *P*–*V* curve can change drastically and in a highly heterogeneous manner (figure 2). Hence, the *P*–*V* curve and lung compliance also reflect the spatial distribution of disease and the interdependence of diseased and normal alveoli.

In general, it is difficult to extract specific information on alveolar remodelling from a single quasi-static lung compliance measurement. When compliance is measured as a function of frequency, the static to dynamic compliance ratio [54] or the variation of compliance or reactance with frequency [55] may convey information about changes in alveolar mechanics. However, it is likely that this is a result of time-constant inequalities involving the spatial distribution of alveolar compliance and airway resistance, without carrying specific insight into the remodelling of individual alveoli [56]. It is therefore useful to supplement pulmonary function tests with CT or MRI, which can reveal regional variations in tissue pathology.

Airway remodelling: structural and functional abnormalities

Origins

A loose definition of airway remodelling is any structural feature of the airway wall which is abnormal in terms of its cross-sectional thickness (area or equivalent volume in three-dimensions) and the composition of its constituent elements. That is, structures may have grown (or shrunk), which in turn alters their mechanical properties. The mechanism(s) of airway remodelling have not been conclusively established, although the majority of scientists favour inflammation as the driver of pathology [57]. A second possibility is structural changes mediated through activation of mechanotransduction pathways, such as that occurring during bronchoconstriction [58]. Finally, evidence that airway remodelling is observed early in life and even prior to diagnosis of obstructive disease [59, 60] raises the possibility that abnormalities of the airway wall are a remnant of some developmental disorder [61]. Even COPD, which has long been associated with cigarette and environmental exposure, is now recognised as having a developmental foundation in some patients [62]. Nevertheless, airway remodelling may also be related to persistent and residual inflammation, even after a stimulus such as cigarette smoke is removed [63].

Structural modifications

There is variation in the broad structure of airways that make up the bronchial tree, where small airways lack cartilage, exhibit more predominant ciliation of the epithelium, and show a greater proportion of club cells and, conversely, fewer mucus-producing goblet cells [64, 65]. The point of distinction between a "large" and "small" airway is often defined as a lumen diameter of <2 mm, a somewhat arbitrary definition that tends to lead to broad hypotheses of small or large airway disease, which may be an oversimplification [66].

At the gross level, airway wall thickness has been shown to be increased in patients with asthma when imaged using CT [67, 68]. While CT can assess total wall thickness, histological assessment is required to resolve specific changes to wall compartments; in patients with asthma, there is an increase in the thickness of the inner and outer airway wall layers and therefore total wall thickness [69]. There is evidence that the airway epithelium is also thicker [70], with hyperplasia of goblet cells [71]. Beneath the epithelium, the basement membrane is thickened in patients with asthma, which is considered one of the most distinguishing features of the disease [72].

Several studies have reported changes to ECM expression within and external to the airway wall in patients with asthma [73, 74]. Changes to ECM within the ASM layer are particularly notable, including an increased expression in elastic fibres and fibronectin within the ASM of large airways from patients with fatal asthma, and an increase in elastic fibres within the ASM of small airways from patients with fatal asthma compared with non-fatal asthma [75].

Another striking change to the airway wall in asthma is an increase in the thickness of the ASM layer. When averaged across cases of asthma, remodelling of the ASM has been observed in both small and large airways [69]. Our understanding of ASM remodelling has since been advanced to consider phenotypes based on anatomical location; remodelling in the small airways or large airways only; remodelling in both the small and large airways; or absence of ASM remodelling altogether [76]. Hence, the presence and extent of ASM remodelling varies both between and within subjects with asthma (figure 3) [77], an observation that should be considered when designing therapies that strive to address these structural abnormalities.

COPD has similarities to asthma in airway remodelling, but also some important differences [78], particularly obliteration of the small airways [79–81]. Again, there is generalised

FIGURE 3 Variation in airway smooth muscle (ASM) remodelling between and within subjects with asthma. Each cluster is a different subject. Each dot is an airway with remodelled (orange) or normal (blue) ASM. FA: fatal asthma; NFA: non-fatal asthma; NA: non-asthmatic. Reproduced and modified from [77] with permission.

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thickening of the airway wall [82], which in some patients includes the ASM layer [83], though perhaps not to the same degree as has been documented in asthma. Variation in ASM remodelling between patients with COPD has also been demonstrated after bronchial biopsy; interestingly, patients with high amounts of ASM responded positively to inhaled corticosteroids compared with patients who had relatively less remodelling [84]. An important distinction in COPD is that the volume fraction of ECM expands disproportionately and is inversely related to lung function [85]; this is in contrast to patients with asthma, where there is a proportional increase in ECM and smooth muscle that accounts for most of the area of the ASM layer [85]. Compositional changes to ECM in COPD are observed throughout the airway wall [86].

Functional changes at the cellular and tissue level

At the cellular and tissue level, structural impairment precedes functional impairment. The degree to which impairment at the local cell or tissue level translates to integrated organ function is dependent upon the distribution and magnitude of the pathology, and airway-to-lung interdependence, as will be discussed. Loss of the epithelial barrier's integrity will increase the sensitivity of the airway wall to a bronchoconstrictor agonist delivered *via* the lumen [87]. Furthermore, the capacity of the epithelium to repair is reduced in both patients with asthma [88] and COPD [89], leaving the airway susceptible to environmental exposures that drive inflammatory processes.

As the principal effector cell producing bronchoconstriction, a change in ASM function is a likely contributor to airflow limitation. Morphology and function of the ASM cells appears intimately associated with the external structural and mechanical environment established by the surrounding ECM. Mechanosensitivity to a specific ECM substrate alters ASM contractile capacity, with evidence of enhanced contraction in the presence of fibronectin [90]. As the stiffness of the external environment to which the ASM is exposed increases, so too does cell size (hypertrophy) and expression of cytoplasmic markers of contractility [91].

At the tissue level (whole airway segments), increased thickness of the ASM layer enhances force production and narrowing in airways from patients with asthma [92]. It has been argued that of all the various changes to the airway wall that characterise remodelling, an increase in ASM thickness is the most influential in driving an increase in bronchoconstriction [93]. Patients with fixed airflow limitation (COPD) exhibit a somewhat different response, with airways narrowing more, even without an increase in gross ASM thickness [94]. An increase in airway narrowing that is independent of ASM bulk is potentially related to the aforementioned changes to ECM within the ASM layer [94] and an associated increase ASM contractility [95].

Functional changes at the end organ level

It has long been understood that functional heterogeneity (namely, bronchoconstriction and heterogeneity of airway calibre) has important consequences for overall lung function; for example, increased heterogeneity of airway calibre, as a result of any of the previously discussed mechanisms that alter function at the cell or tissue level, leads to elevated resistance and elastance of the whole system [96, 97]. Paired with the notion that functional heterogeneity could be self-organised, that it arises from a structurally homogeneous beginning [98, 99], it is tempting to conclude that structural heterogeneity (*e.g.* of ASM thickness) is a secondary consideration. While it may be the case that this dynamic (or self-organised) heterogeneity is present *in vivo*, it is also certainly true that there is significant structural heterogeneity [77], and that overall patterns of functional heterogeneity are likely to emerge from a combination of the underlying structural heterogeneity working in concert with the dynamic self-emergence process [100]. This is supported by the observation that the locations of ventilation defects in asthma are largely, though not entirely, persistent over time [101].

Everything being equal, increased structural heterogeneity should lead to decreased lung function and increased temporal persistence of ventilation patterns [102]. However, this is an oversimplification, and potentially a gross one, because there are many ways in which airway structure can be heterogeneous. The simplest notion is to imagine that airway structure is independent and uncorrelated, in which case simple measures of dispersion (*e.g.* coefficient of variation) might suffice. In reality, that does not appear to be the case; there are significant correlations in remodelled airway structure both "along" and "across" flow pathways [77], and these correlations may have significant implications for resulting functional heterogeneity and lung function. The mechanism through which these structural correlations are generated is unclear, which is perhaps unsurprising given that the mechanism of remodelling itself is disputed. However, some possibilities have been suggested [103], and while the correlation in structure itself is only partially revealed, the fact that airway remodelling is correlated may offer a clue as to the underlying remodelling process itself. Moreover, the precise ways in which structural heterogeneity, in its many guises, manifests as functional heterogeneity, remains only partially solved [104].

The above largely focusses on the heterogeneity of ASM thickness, and airway calibre, as prototypical examples of structural and functional heterogeneity, respectively. There are, of course, other important aspects that manifest heterogeneously; for example, the location and extent of mucus plugs [105, 106]. The statistics and correlations of the location of these plugs are not perfectly understood but will seemingly have important contributions to overall functional heterogeneity.

Length adaptation: a smooth muscle property that may underlie some of the unique functions and dysfunctions of the lung

Smooth muscle has a working length range that is much greater than that of skeletal or cardiac muscle. This is essential for the proper function of hollow organs, like the stomach, bladder, uterus and digestive tract that regularly undergo large volume changes. The same property, when not regulated appropriately, could be a source for pathophysiology that underlies diseases like asthma and hypertension. To understand smooth muscle-related organ dysfunction, one needs to know the mechanism responsible for the muscle's ability to retain contractility over a large length range.

When a striated muscle is stretched over 60% from its optimal length, it will not be able to generate active force because the actin and myosin filaments no longer overlap each other [107]. In contrast, smooth muscle lining the urinary bladder is able to generate force over a seven-fold length range [108]. In ASM, a constant active isometric force can be maintained over a three-fold length range [109]. To achieve this, the muscle relies on a mechanism called "length adaptation" [110].

A simple definition of length adaptation is that it is a process initiated by a change in cell length, which involves rearrangement of contractile and cytoskeletal filaments to ensure maximal overlap between actin and myosin filaments, in order to maintain optimal force generation. The process does not occur instantly after a length change; rather, it takes some minutes. There is evidence that immediately following a length change, the ability of ASM to generate force is reduced, which accompanies partial dissolution of myosin filaments [111]. When the length of the muscle stops changing, the muscle contractility recovers over time (20–30 min, with periodic stimulation), along with repolymerisation of myosin filaments [111]. This myosin filament evanescence is part of a much more complex process, in which dismantling

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and rebuilding of the contractile apparatus occurs in adaptation to a change in cell geometry [61]. In ASM cells, there is evidence that myosin filaments and myosin monomers or dimers are in equilibrium in a dynamic polymerisation and depolymerisation process, facilitated by the existence of a pool of myosin monomers and dimers within the cytoplasm of smooth muscle cells [112, 113].

Functional evidence indicates that in ASM adapted to different lengths, shortening velocity, power output and adenosine triphosphate consumption of the muscle are linearly proportional o the cell length, whereas isometric force is length-independent [114]. To explain the observations, a conceptual model of length adaptation has been proposed, as illustrated in figure 4. The increased shortening velocity in ASM adapted to a longer length can be explained by the increased number of contractile actomyosin units in series. The increased power output and energy consumption can be explained by the increase in the overall number of contractile units in the muscle adapted to a longer length. The length-independent isometric force at different adapted lengths can be explained if length adaptation involves only addition and subtraction of contractile

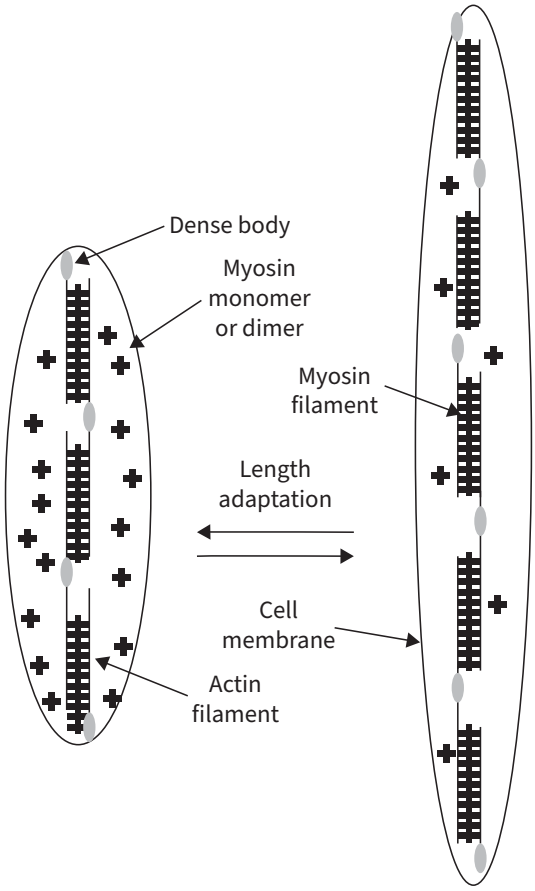

FIGURE 4 Reversible length adaptation in smooth muscle. When a muscle cell is lengthened or shortened from its original adapted length, disassembly of the contractile apparatus occurs, resulting in a loss of contractility. This is followed by reassembly of the contractile apparatus at the new cell length, so that the overlap between the myosin and actin filaments is optimal. This reassembly phase is accompanied by the recovery of the muscle's ability to generate force. The dynamic equilibrium between myosin filaments and myosin monomers and dimers facilitates the adaptation process. Note that a key feature of the adaptation process is that the contractile units can only be added or subtracted from the contractile unit array in series. This is required to accommodate the length-dependent behaviour of the muscle in terms of force, velocity and power, as described in the main text.

units in series, without changing the number of contractile units in parallel. Besides functional changes, structural changes in ASM adapted to different lengths have also been found. Using the number of myosin filaments in a cell cross-section (myosin filament density) to indicate the extent of myosin filament polymerisation, it has been shown that filament polymerisation rises as the adapted muscle length increases [114], supporting the assumption that more contractile units are present in smooth muscle cells adapted to longer lengths. The model shown in figure 4 presents a minimalist approach to explaining smooth muscle structure and function. As more structural and functional properties of smooth muscle are uncovered, more complexity will need to be added to the model to accommodate new evidence.

A shift in the length–force relationship of ASM is another consequence of length adaptation [111]. As illustrated in figure 5, adaptation of ASM to lengths shorter or longer than the reference length results in shifts to the left or right of the length–force curve respectively, while maintaining maximal force. In striated muscle, the length–force relationship cannot be shifted, because the underlying sarcomeric structure in striated muscle is fixed [107]. The fact that the same relationship in smooth muscle can be shifted indicates that the structure of the contractile apparatus in smooth muscle is not fixed, and can be altered by length adaptation. When fully adapted, ASM can generate maximal force. However, immediately after a length change (either shortening or lengthening), ASM can only generate submaximal force defined by the specific length–force curve associated with a specific length at which the muscle has been adapted (figure 5).

The length adaptation model described in figure 4 is compatible with the argument that smooth muscle cells possess material properties similar to that of plastic [109] or soft glass [115]. The model predicts that dissolution of contractile filaments in smooth muscle cells occurs after a change in cell length, which can be lengthening, shortening or length oscillation. During this phase of length adaptation, smooth muscle cells temporarily lose their ability to generate maximal force. It is likely that this is an explanation for the bronchodilatory and bronchoprotective effect of DI. The DI effect has been observed in human subjects [116, 117] and in *ex vivo* lungs [118, 119]. Furthermore, the distension of airway calibre during DI has been shown to be

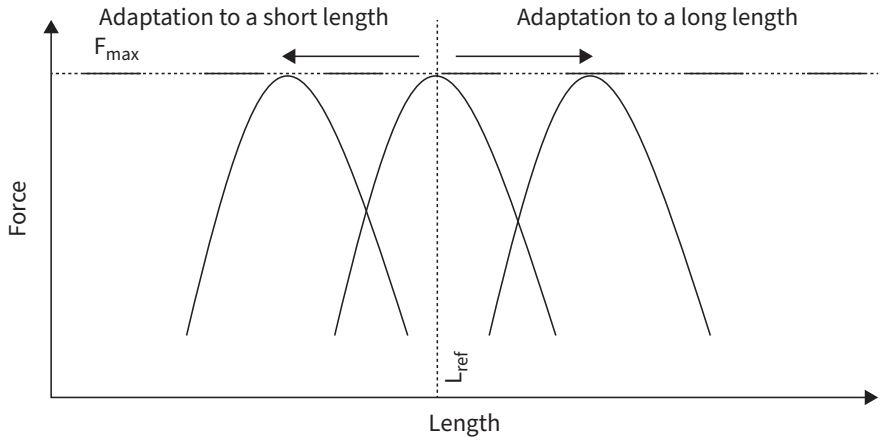

FIGURE 5 Shifting the length–force relationship due to length adaptation. After the muscle has been fully adapted to a reference length (L_{ref}), shortening or lengthening from L_{ref} will result in a temporary loss of contractility, along the curve specific to the muscle adapted to L_{ref}. However, if the muscle is held at the shortened or lengthened state for a prolonged period of time (*e.g.* 30 min), the curve will shift with little change in shape, so that the muscle can again generate the maximal force (F_{max}). The shift can be explained by the model shown in figure 4.

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sufficient to stretch the smooth muscle cells encircling the airways and cause a temporary loss of contractility of these cells [119, 120]. These observations provide a plausible explanation for the bronchodilatory and bronchoprotective effects of DI.

The lack of beneficial effects of DI in asthmatic patients [117] could be a consequence of maladaptation of ASM to an abnormally short length, causing it to enter a "frozen state" [121], meaning DIs are unable to "fluidise" [122]. In human asthmatic lungs, it has been noted that ASM cells are hyperreactive [123]. This could be a consequence of "force adaptation"; that is, an increase of ASM contractility due to chronic activation of the muscle cells in the inflammatory environment of the asthmatic lung [124]. Chronic activation of ASM could put the muscle in a frozen state and become refractory to DI perturbation.

Airway hyperresponsiveness develops when lung volume is chronically reduced (for example, in cases in which chest strapping is used [125], when the subject is in the supine position [126] or when the subject is obese [127]). In such situations, ASM may adjust to abnormally short lengths. As illustrated in figure 5, maximal force can be achieved again after adapttion to a short length, resulting in exaggerated airway narrowing. When lung volume is increased, such as under positive end-expiratory pressure or continuous positive airway pressure, experimentally induced bronchoconstriction in healthy human subjects can be attenuated and airway reactivity in stable asthmatics can be reduced [128]. These can be taken as examples of adapting ASM at long lengths. As illustrated in figure 5, when ASM is adapted to a long length, shortening of the muscle results in less force generation (without adaptation), thus conferring bronchoprotection on the lung. Knowing the adaptive behaviour of smooth muscle, adaptation of ASM to short lengths should be prevented to preserve healthy lung function.

The impact of airway-to-parenchymal interdependence on lung function tests

In vivo, the airflow through the airways is critically dependent on the balance between factors that favour and factors that contrast with the airway tone [129, 130], the former being the force-generation capacity of ASM and the latter being the magnitude of internal and external elastic loads (figure 6). However, due to the complexity of lung structure-to-function changes in disease, their net effect on airway calibre may be highly variable and even contrary to expectations.

In this section of the chapter, we will examine how the mechanisms and structural characteristics that modulate airway narrowing may impact on standard lung function tests in health and disease.

The effects of lung inflation on airway calibre

Following the seminal work by Nadel and Tierney [116], which reported that DI temporarily reduces the resistance of constricted airways *in vivo*, a long series of studies confirmed that this phenomenon is consistent in healthy subjects but absent or minimal in asthmatic subjects. The lack of relationship between this and indices of airway inflammation [131] suggests that the impaired effect of DI in asthma is the result of reduced distensibility of the airway walls, possibly because of an increase in ASM and abnormal length adaptation, airway wall remodelling, or reduced stretching by the surrounding lung parenchyma [132, 133]. Whatever the mechanism, the different effects of DI between healthy and asthmatic subjects have an important impact on the clinical assessment of airway responsiveness, with spirometric measurement that requires a DI becoming more sensitive in separating asthmatic from non-asthmatic subjects than measurements performed during tidal volume, such as airway resistance [117, 134]. In 1995, Skloot *et al.* [135] found similar methacholine dose–response

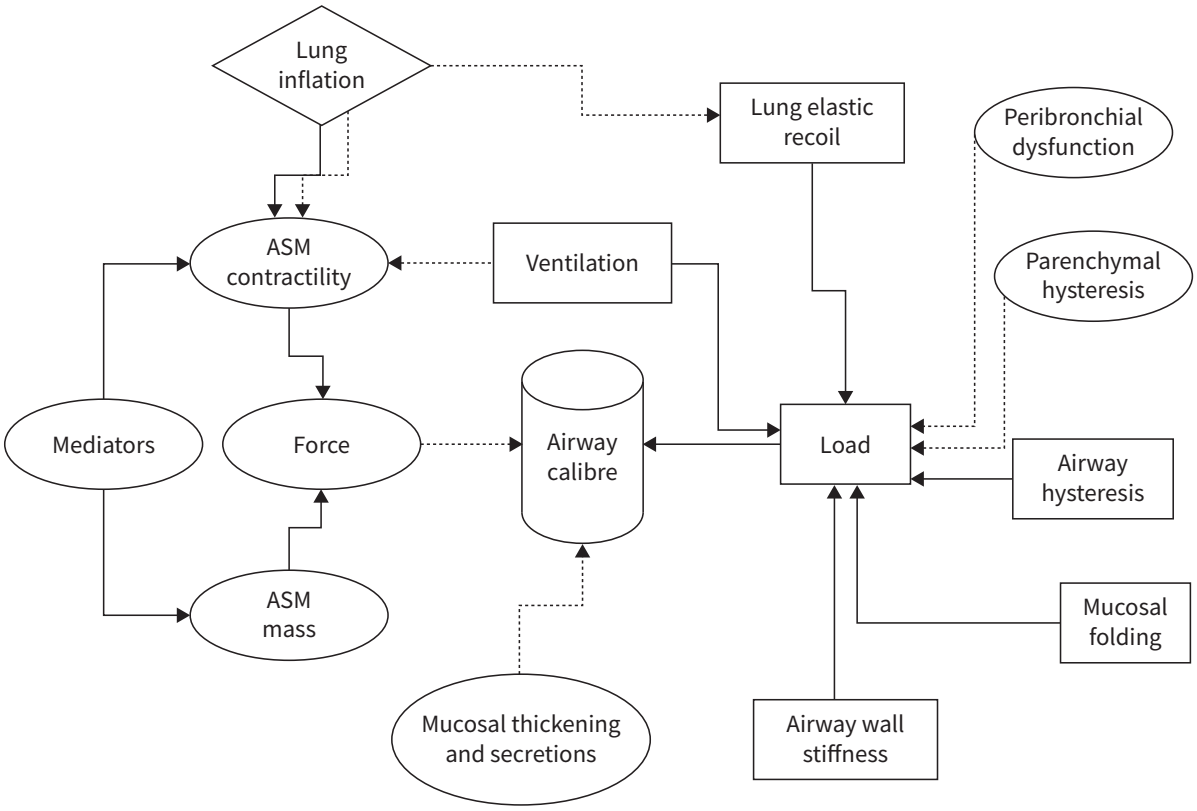

FIGURE 6 Schematic representation of factors favouring (in ovals) and opposing (in rectangles) airway narrowing. Continuous and dotted lines denote increasing and decreasing effects, respectively. Airway smooth muscle (ASM) force together with mucosal thickening and bronchial secretions are the mechanisms that directly decrease bronchial lumen. In contrast, the loads external and internal to the airway wall are the mechanisms that tend to limit ASM shortening, thus opposing airway narrowing. Note that lung inflation has a negative effect on load, as it transiently decreases elastic recoil, and an unpredictable effect on ASM, as it is capable of decreasing ASM contractility in health, but increasing it in asthma. Reproduced and modified from [129] with permission.

curves in asthmatic and normal subjects when DIs were strictly prohibited. This supports the idea that hyperresponsiveness in asthma may reflect an inability to dilate constricted airways rather an increased response to stimuli [135]. This was only partially confirmed when volume-independent measurements were used, suggesting that both impaired bronchodilation and increased contractile response may contribute to airway hyperresponsiveness [131]. A bronchoprotective effect of DIs taken before a single dose of methacholine was observed in healthy but not asthmatic subjects [136]. However, the bronchoconstrictor response inferred by measurements that do not require full lung inflation was enhanced rather than attenuated by prior DIs both in healthy and asthmatic subjects [137].

Several studies suggest that the effects of DI on the assessment of lung function are widely variable. In asthmatic subjects with spontaneous bronchoconstriction [138] and in COPD [139], forced expiratory flows were found to be lower during a manoeuvre beginning at maximal rather than partial lung inflation, which was explained by a discrepancy between the hysteretic properties of airways and lung parenchyma [132, 138, 140]. The practical consequence of this short-lasting bronchoconstrictor effect is that the effect of bronchodilators may be underestimated by classical spirometric measures [141]. In COPD subjects with extensive emphysema, FEV_1 may not increase after bronchodilator inhalation, despite an increase in FVC. The potential mechanisms for this isolated volume response are: space competition between

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airways; distended alveoli; and a decrease in airway calibre with lung inflation due to longitudinal stretching in the presence of reduced radial tethering force [141]. Finally, in a small subset of asthmatic subjects, a decrease in FEV_1 was reported to occur with subsequent spirometric manoeuvres, which was explained by an increase in ASM tone caused by DI triggering a myogenic mechanism [142–144].

Another important mechanism linking DI to lung function assessment in clinical practice is intrathoracic gas compression. During forced expiration, alveolar pressure increases, and gas is compressed within the lung, thus causing lung volume and lung elastic recoil to decrease. The latter will result in a reduction of driving pressure and distending pressure at choke point, thus explaining why FEV_1 measured at the mouth is less than it would be if measured by body plethysmography. The magnitude of this effect is determined by the amount of gas within the lung and airway resistance. Practical consequences are that FEV_1 tends to overestimate the severity of airflow obstruction in emphysema [145] and airway responses to pharmacological interventions in subjects with larger lungs [146].

The effects of breathing pattern and operational lung volume

Studies in animals and humans have provided clear evidence that increasing the operational lung volume or tidal volume reduces airway resistance and can attenuate or reverse the response to bronchoconstrictor stimuli.

In vitro, dynamic swings can blunt the response of ASM to contractile stimuli through mechanisms that reduce its force-generation capacity [147, 148], depending on the magnitude and frequency of pressure oscillations. In healthy humans, bouts of five tidal volumes terminating at ∼80%, ∼90% and 100% of TLC have been shown to cause gradual bronchodilation that lasts for ⩾11 min [149]. Increasing ventilation by breathing a CO_2-enriched mixture [150] or through hypobaric conditions [151] significantly blunts the bronchoconstrictor response to methacholine.

Breathing voluntarily at increased lung volume has also been shown to attenuate induced bronchoconstriction in healthy subjects [152, 153]. However, the major determinant appeared to be the operational lung volume, whether attained by increasing FRC or tidal volume [153]. Conversely, breathing voluntarily at low lung volume enhanced the bronchoconstrictor response [152]. Analogously, airway responses to bronchoconstrictor stimuli are enhanced in healthy subjects using chest strapping [125], in the supine position [126] and in obesity [127], with all conditions associated with breathing at low lung volume.

Collectively, the available literature supports the notion that changes in lung volume are strong a mechanism of airway calibre modulation. However, while increasing operational lung volume has a beneficial effect that opposes airway narrowing, the effects of DI may vary according to disease conditions.

The effects of ventilation heterogeneity: topographical and temporal patterns

It has long been understood that ventilation is not uniform in obstructive lung diseases [154], but its impact on global lung function tests was not investigated until the beginning of this millennium, when imaging studies documented the occurrence of large and important ventilatory defects during bronchospasm [98, 155, 156].

Integrative modelling approaches suggested that even minimal anatomical variations between peripheral airways of the same generation could cause responses to a constrictor agent that

differed in magnitude and time, resulting in airflow diverting from more to less constricted airways, thus paradoxically increasing the size of the latter (parallel inhomogeneity) [99]. This mechanism may explain the insensitivity of forced expiratory flows to lung inhomogeneity during induced bronchoconstriction [157]. Similar results were also achieved by considering the hypothesis that constriction of more central airways will cause hypoventilation of the subtending regions (serial heterogeneities), thus magnifying the effects of parallel heterogeneities occurring within the peripheral airways [155]. Two major studies then attracted the attention of the medical community on this issue. The first demonstrated that replications of such events were associated with the occurrence of severely hypoventilated regions once a given threshold of variability was exceeded [98]. The second found that ventilation heterogeneity was a major determinant of airway hyperresponsiveness in asthma, independent of airway inflammation [158]. However, in a recent study [159] airway hyperresponsiveness and abnormalities of single- or multiple-breath nitrogen washout [160] were frequently present in subjects with suspicion of asthma and normal spirometry, but were not necessarily associated in individual subjects. This lack of concordance suggests that these tests reflect different pathological aspects of the disease.

Ventilation heterogeneity has also been examined under the time domain on the ground that the respiratory system exhibits mechanical fluctuations over different time scales. In a seminal study by Que *et al.* [161], the variability of respiratory impedance measured using the forced oscillation technique for 15 min was much higher in asthmatic individuals than in healthy subjects. When the latter inhaled a constrictor agent in supine position, the impedance variability became similar to that of asthmatics, confirming the idea that ASM contraction and unloading are basic mechanisms modulating bronchial tone. Importantly, the impedance time series exhibited power law features with similar exponent of the relationship between groups, suggesting that that the phenomenon is replicable at different time scales. The study by Que *et al.* [161] paved the way for the investigation of temporal variability, which represents a major characteristic of bronchial asthma. Studies using peak expiratory flow were able to predict asthma attacks 1 month in advance [162], as well as asthma worsening after withdrawing steroid therapy [163]. Monitoring the daily variability of oscillatory resistance not only allowed the prediction of airway narrowing 1 week in advance [164] but also made it possible to separate asthmatic from healthy subjects more accurately and over a shorter period of time than by monitoring peak expiratory flow [165].

Based on the above studies, it is hoped that the topographical and temporal variabilities of airway narrowing will take a crucial place in the armamentarium of fundamental markers for asthma diagnosis and monitoring in clinical practice.

Conclusion

In health, lung function is optimised by the mechanical interaction between airways and lung parenchyma. In disease, heterogeneous structural changes occur in both the lung parenchyma and the airways, impairing the ability of the whole organ to maintain a normal homeostatic state in response to external stimuli. In this context, an important role is played by the ASM length-adaptation property, with an abnormally short length placing the muscle in a “frozen” state, making it unresponsive to the broncho-relaxant effect of deep and tidal inspirations. Owing to the complex interactions between various structural units of the whole organ, the mechanical effects of breathing can either enhance or attenuate the functional impairment depending on the type and severity of underlying disease. This means the interpretation of lung function tests in clinical practice is not always univocal, nor is it compliant with the expectations and/or the recommendations of international guidelines.

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