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INVITED REVIEW SERIES: RESPIRATORY DISEASE: USING LUNG FUNCTION MEASUREMENTS TO GREATER ADVANTAGE
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Asthma and respiratory physiology: Putting lung function into perspective

GIUSEPPE FRANCESCO SFERRAZZA PAPA,[1] GIULIA MICHELA PELLEGRINO[1] AND RICCARDO PELLEGRINO[2]

[1]*Respiratory Unit, San Paolo Hospital, University of Milan, Milan and* [2]*Allergology and Respiratory Physiopathology, Santa Croce e Carle Hospital, Cuneo, Italy*

ABSTRACT

Bronchial asthma is a chronic disease characterized by airway hyperresponsiveness, airway inflammation and remodelling. The hypothesis that the illness is inflammatory in nature has recently been challenged by studies showing that airway smooth muscle (ASM) plays a more important role than previously thought. For example, it is now known that in asthma patients, ASM proliferates more and faster than in healthy subjects, carries intrinsic defects and exhibits impaired relaxation, increased velocity of shortening, plastic adaptation to short length and perturbed equilibrium of actin-to-myosin during cycling. Similar conclusions can be drawn from studies on airway mechanics. For instance, in asthma, abnormal ASM contributes to limiting the response to deep lung stretching and accelerates the return of bronchial tone to baseline conditions, and contributes to increased airway stiffness. Upon stimulation, ASM causes airway narrowing that is heterogeneous across the lung and variable over time. This heterogeneity leads to patchy ventilation. Experimental studies have shown that patchy ventilation may precipitate an asthma attack, and inability to maintain bronchial tone control over time can predict the occurrence of bronchospastic attacks over a matter of a few days. To improve our knowledge on the pathogenesis of asthma, we believe that it is necessary to explore the disease within the framework of the topographical, volume and time domains of the lung that play an important role in setting the severity and progression of the disease. Application of the forced oscillation technique and multiple breath nitrogen washout may, alone or in combination, help address questions unsolvable until now.

Key words: asthma, airway smooth muscle, forced oscillation technique, remodelling, respiratory function test.

Abbreviations: AHR, airway hyperresponsiveness; AR, airway responsiveness; CPAP, continuous positive airway pressure; DB, deep breath; FOT, forced oscillation technique; FRC, functional residual capacity; M/P ratio, ratio of maximal-to-partial forced expiratory flows; MBNWO, multiple breath nitrogen washout; MCh, methacholine; PEF, peak expiratory flow; R5, inspiratory resistance at 5 Hz; R5CV, coefficient of variation of R5; RV, residual volume; Zrs, respiratory impedance.

BRONCHIAL ASTHMA: A COMPLEX DISEASE

Bronchial asthma is a chronic disease characterized by airway hyperresponsiveness (AHR) to a series of allergens or irritants.[1] Coexistence of airway inflammation and remodelling has led to the hypothesis that the disease is inflammatory in nature,[1] although the relationship between the inflammatory process and AHR has not been consistently shown.[2] For example, studies examined by Brusasco *et al.*[3] revealed that markers of inflammation correlated with AHR in no more than 50% of the cases. In addition, pharmacological interventions have shown that inflammation can be modified independently of AHR and vice versa.[2] It is significant that, despite a multitude of studies on cellular and molecular lung biology and immunology, the precise pathogenesis of asthma still remains unknown. In this review, we examine the disease from a different perspective. Specifically, we suggest that the pathogenesis of asthma can be better understood if we start from the principle that the lung is a complex organ and any chronic disease affecting it will also become complex.

Correspondence: Riccardo Pellegrino, Allergologia e Fisiopatologia Respiratoria, ASO S. Croce e Carle, 12100 Cuneo, Italy. Email: pellegrino.r@ospedale.cuneo.it

The Authors: Giuseppe Francesco Sferrazza Papa, MD, is a Fellow in Respiratory Medicine with interest in chronic obstructive pulmonary diseases, lung function and imaging techniques. Giulia Michela Pellegrino, MD, is a Fellow in Respiratory Medicine with interest in chronic obstructive pulmonary diseases, lung function and clinical research. Riccardo Pellegrino, MD, is Chief of the Department of Allergology and Respiratory Pathophysiology with interest in chronic obstructive pulmonary diseases, lung and airway mechanics, airway hyperresponsiveness and exercise physiology.

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LUNG FUNCTION IN ASTHMA

Historical view

Most of the lung function studies conducted in the 1980s and 1990s focused on two main features of the disease—peak expiratory flow (PEF) variability and airway narrowing pattern. Monitoring of PEF indicated that well-being periods in asthma patients are typically interspersed with bronchospastic attacks. As such, PEF measurement was included in the list of useful tests for diagnosis and follow up of the disease.[4] Despite the assessment of PEF variability not achieving a widespread position in clinical practice for a variety of reasons, the concept that the lung function of asthma patients fluctuates over time has been regaining interest with respect to the underlying disease mechanisms.[5–8]

Description of lung function during an attack has historically included a decrease in flow and airway conductance.[9] Relating the two parameters to lung elastic recoil has provided evidence that the functional abnormalities in asthma were due to intrinsic airway narrowing rather than loss of lung elasticity. These changes have also been associated with an increase in functional residual capacity (FRC) and an increase in respiratory elastic work of breathing, which is compensated by a decrease in airflow resistance. An increase in residual volume (RV) mirrors the decrease in vital capacity, thus implying the occurrence of airway closure, a marker of disease severity. The localization of the site of airway narrowing using gas with different density has also been investigated, with studies suggesting that there is no preferential site of narrowing and either peripheral or central airways may undergo narrowing.[10] Another major historical finding was the recognition that ventilation was unevenly distributed in asthma.[11] Identification of such uneven distribution as a means to detect early signs of the disease, even when lung function is still within or near normal range,[9] has formed the basis for much of the current research focus in asthma.

Lung function in bronchial asthma: new perspectives

Modelling airway responsiveness

Throughout the 1990s and the 2000s, the dominant opinion was that asthma was an inflammatory disease. This view was based on studies documenting an association between AHR and inflammation and remodelling within the airway wall.[1] Leftward shifts of the bronchial dose–response curves were interpreted as a result of prejunctional factors such as epithelial damage, inflammatory cells and mediators, and neural stimuli facilitating the initial response. In contrast, post-junctional mechanisms such as airway smooth muscle (ASM) contractility, lung elastic loads and wall thickness were thought to explain the loss of plateau and exaggerated response.[12]

The inflammatory theory has been challenged by studies reporting no or insubstantial relationships between lung function and morphology.[3,13] Further, new computational models that assess airway and lung mechanics with a variable number of parameters and levels of interaction[14–21] have shown that AHR is the result of complex interactions among ASM, remodelling, inflammation, load and breathing pattern,[2] although many of the functional features have been ascribed to ASM. The role of the latter is supported by studies in asthma patients showing that ASM proliferates more[22] and faster than in healthy subjects,[23,24] may carry intrinsic defects[23–27] and exhibits impaired relaxation,[28] increased velocity of shortening,[23] plastic adaptation to short length[17] and perturbed equilibrium of actin-to-myosin during cycling.[15,16] Further support for the notion that ASM plays a primary role in asthma is based on the rapid effects of β_2 stimulants to relieve bronchospasm[9] and the beneficial effects of thermoplasty on airway responsiveness.[29] In summary, these studies have contributed to the idea that ASM is not simply an effecter of the asthmatic response, as previously thought, but that it exhibits intrinsic abnormalities leading to greater shortening and contributing to the orchestration of inflammation and remodelling.

Assessing the determinants of airway narrowing in vivo

Over the last few decades, new and unconventional functional tests capable of exploring airway mechanics have been introduced to examine more closely airway narrowing in asthma. These tests have focused on exploring airway mechanics within the volume and time domains.

The effects of deep breath on bronchomotor tone. Nadel and Tierney were the first to report that taking a deep breath (DB) in healthy subjects exposed to a constrictor agent was associated with a significant decrease in airway resistance.[30] This observation motivated a number of investigations, the results of which can be summarized as follows.[31] In healthy subjects exposed to any constrictor agent, DB causes an increase in flow and/or airway conductance, and a decrease in RV. The bronchodilator effects last for 1–2 min. In contrast, in asthmatics such increase in flow or airway conductance and decrease in RV are remarkably blunted depending on the severity of narrowing.[32–35] Compared with healthy controls, the airways reconstrict at a faster rate.[36–40] In very severe airflow obstruction, DB may even cause transient bronchoconstriction as documented by a decrease in flow and/or airway conductance.[37] In a minority of asthmatics, a DB at baseline conditions results in a reduction in maximal flow and airway conductance that worsens with the next large breaths.[41] This response appears to be sensitive to calcium blockers, a mechanism attributed to a myogenic reflex triggered by lung inflation. The findings from these studies have resulted in several new theories to explain bronchospasm.

Ingram formulated the hypothesis that the ratio of maximal-to-partial forced expiratory flows (M/P ratio) may reflect the site and nature of the disease.[42] His reasoning was based on the observation that, in

spontaneous asthma, DB causes bronchoconstriction,[35] whereas it causes bronchodilatation in induced bronchospasm.[32,36,39] These findings were interpreted according to the Froeb and Mead theory that relates airway-to-parenchyma physical properties.[43] In brief, on inspiration the airways dilate because of the tethering effects of lung elastic recoil. On expiration, both airways and lung parenchyma dissipate part of the energy stored on inspiration, as shown by the looping of their pressure–volume relationship (hysteresis). If pressure dissipation is the same for airways and lung parenchyma, then airway calibre and thus flow at a given lung volume will be the same on inspiration and expiration (Fig. 1, upper panels). If airway hysteresis exceeds parenchymal hysteresis, the constrictive pressure will decrease relative to lung recoil (Fig. 1, middle panels). This will cause airway calibre to be larger on expiration than on inspiration, and the opposite will happen when parenchymal hysteresis exceeds airway hysteresis (Fig. 1, lower panels). According to this theory, an increase in the M/P ratio is attributed to a larger pressure dissipation within the airway contractile tissues. Conversely, a reduction of M/P ratio is due to a larger decrease in parenchymal than airway hysteresis with a full inflation. This might be caused by increased airway wall stiffness, preventing the airways from distending with large breaths or by peripheral lung involvement. This theory has found support in two studies. The first documented

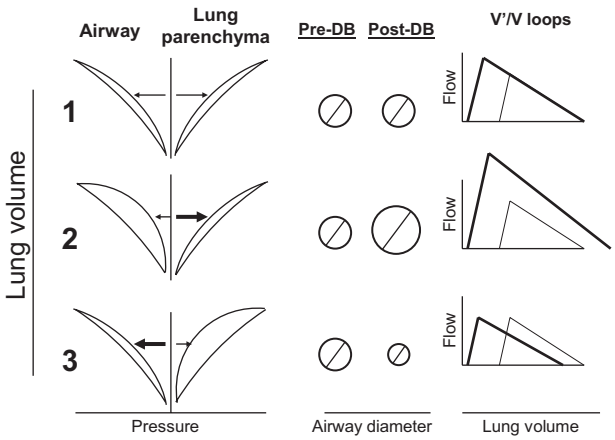

Figure 1 Schematic representation of the relative hysteresis theory. On the left, pressure–volume curves of the airways and lung parenchyma during maximum inflation and deflation are presented for three different conditions. Bronchial diameter reflects the balance between airway and lung tensions, which have constrictor and dilating effects, respectively. On the right, expiratory flow–volume curves before (partial loop) and after a deep breath (maximal loop) are shown. Case 1: airway and parenchymal hystereses are the same. For a given lung volume, airway and lung recoils will be the same and so partial and maximal flows. Case 2: airway hysteresis exceeds parenchymal hysteresis. As a result, on expiration lung elastic recoil will be greater than bronchial pressure so that airway calibre and thus flow will increase after a deep breath. Case 3: parenchymal hysteresis is larger than airway hysteresis. Lung recoil will be less than airway pressure during deflation. As a result, airway size and flow will decrease after deep breath. DB, deep breath.

an increase in parenchyma hysteresis in asthmatics undergoing bronchial challenge compared with healthy controls under similar conditions.[33] The second provided evidence that if narrowing exceeds a given threshold in asthma, then the M/P ratio decreases as a result of an increase in parenchymal hysteresis.[32] The blunted response to DB in asthma might also be explained by airway inflammation on the basis of a negative relationship between the M/P ratio and bronchoalveolar lavage eosinophils and proteins.[35] The presence of exudates and inflamed tissues would render the airways resistant to the external load, thus causing less decrease in bronchial than parenchymal tone with DB. Additional studies appear to support this line of reasoning. For instance, in allergic asthmatics, constriction occurring late during an allergen bronchial challenge has been shown to be associated with a decrease in the M/P ratio compared with an early constrictive response.[44] As allergen-induced asthmatic reaction is associated with airway inflammation,[45] the inflammation response was interpreted as a mechanism contributing to the stiffening of the bronchial wall, thereby rendering it little responsive to DB. In naïve asthmatics, a course of inhaled steroids causes a significant increase in the M/P ratio during a methacholine (MCh) challenge,[46] as if reducing airway wall thickening, secretions and peribronchial oedema allowed the airways to distend more with DB than in asthmatics before treatment. In two groups of asthmatics, one with limited and the other with exaggerated response to MCh, Pellegrino *et al.*[47] showed that the inability of the airways to respond to a DB was related to exaggerated response to the constrictor agent, this being the result of airway wall remodelling, loss of airway-to-parenchyma interdependence or ASM intrinsic abnormalities. The airway and lung parenchyma hysteresis theory provides a conceptual framework for the interpretation of the effects of DB in asthma as it states that in asthma any increase in ASM mass or airway wall thickness or decrease in lung elastic recoil may reduce, alone or in concert, the effects of lung distension on airway mechanics. However, this theory does not clarify the mechanisms through which this might occur at the cellular and molecular levels.

It was not until the study of Skloot *et al.*[48] that ASM was recognized as a major determinant of bronchospasm in asthma. This seminal study examined the response to MCh in a group of asthmatics and healthy controls when DB were or were not taken.[48] Interestingly, when the challenge was conducted without DB, the response to MCh in the controls became indistinguishable from that of the asthmatics. It was as if healthy subjects became asthmatic simply by avoiding DB. Ruling out any role of airway inflammation and remodelling to interpret the results, the authors concluded that the ASM precipitated the response to MCh. Subsequent and independent studies conducted with a similar protocol but with measurements more sensitive to airway size than those used by Skloot *et al.* have questioned the broad conclusions of this study[49,50] and suggested that other mechanisms probably contribute to AHR in asthma. In addition, Kapsali *et al.* speculated that AHR in

asthma might also be due to the loss of a natural bronchoprotective mechanism.[51] That is, if a bronchial challenge in healthy subjects is preceded by a series of DB, then the ensuing response to the active agent is less intense than when no DB are taken—and that this mechanism is missing in asthmatic patients. The study was conducted with the use of the forced expiratory volume in 1 s to estimate the changes in airway function with MCh. Subsequent studies conducted in humans with the DB-independent measurements have not reproduced these results; furthermore, showing that in healthy, rhinitic and asthmatic subjects a series of DB preceding the challenge was paradoxically associated with narrowing.[52] Today, the precise role of bronchoprotective mechanisms in the pathogenesis of asthma is a matter of debate.

Lutchen *et al.* hypothesized that the inability of DB to dilate the airways in asthma was the result of heterogeneous distribution of airway narrowing within the lungs.[38] Indeed, inflammation and remodelling contribute to render narrowing extremely heterogeneous across the lungs with some airways becoming fully closed or near closure during a bronchial challenge. A similar pattern occurs in healthy subjects exposed to a constrictor agent, although to a lesser extent compared with asthmatics. In healthy subjects, a DB relieves airway narrowing and closure because the tethering forces of the lung inflation are promptly transmitted to the airways, thus increasing size and recruiting hypoventilated regions. In contrast, in asthmatic patients, airway inflammation and remodelling contribute to amplifying the heterogeneities and severity of airway response across the lungs so that more airways become fully closed. Under these conditions, a DB proves less effective in opening and recruiting closed lung regions as distribution of ventilation is much more heterogeneous compared with lung elastic recoil.

In healthy subjects exposed to a constrictor agent, the decrease in airflow resistance after a DB is followed by a gradual return to baseline condition within 1–2 min.[36,37,39,40,53–55] In asthma, airway reconstriction is much faster and is dependent on the severity of the disease.[39] Whether this behaviour contributes to the severity of asthma is unknown, although this prompt airway reconstriction might be a reflection of an increased ASM velocity of shortening.[56] As documented in isolated ASM from sensitized animals, shortening is faster than in control upon stimulation.[57] The proposed link between increased velocity of shortening and AHR is schematically shown in Fig. 2. Under normal conditions, ASM synchronously adapts to the changes in tidal breathing so that airway size slightly increases on inspiration and returns to the pre-inspiration state on expiration. An increase in ASM velocity of shortening would cause faster shortening during expiration so that airway size at the end of expiration will be smaller with respect to the previous breath and will continue to decrease over the subsequent breaths until wall tension is fully counterbalanced by parenchymal pressure. Conditions such as inflammation and/or allergy exposure that are known to increase ASM velocity of shortening

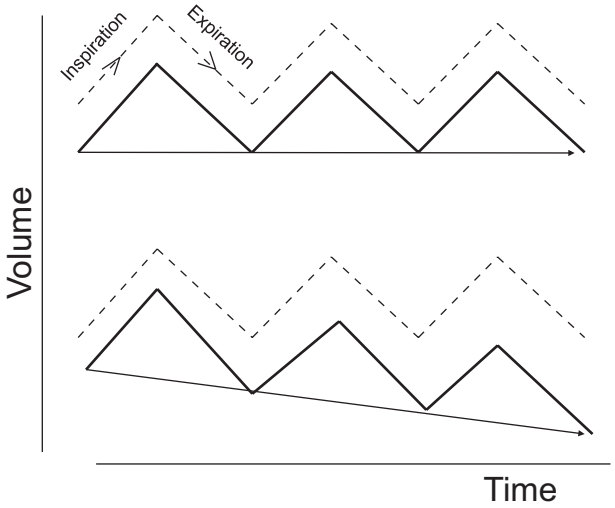

Figure 2 Schematic representation of the effects of increased velocity of airway smooth muscle (ASM) shortening on airway size. Lung and airways volumes (dashed and continuous lines, respectively) are plotted versus time during tidal breathing. Upper panel: airway volume synchronously increases with the increase in lung volume and then returns to baseline values at each breath (normal conditions). Lower panel: airway volume increases with the increase in lung volume, but then achieves a lower volume at the end of expiration because of an increased ASM shortening. As a result, airway narrowing gradually occurs.

presumably contribute to explaining the observed increments in AHR in chronic asthma or during exacerbations. Yet, as ASM shortening depends on the passive wall tension and parenchymal load, any faster renarrowing *in vivo* in humans might not necessarily represent a unique marker of velocity of shortening.

As discussed above, a single DB is often ineffective in relieving bronchial tone and dyspnoea in asthma patients. However, increasing the number and depth of tidal breaths has been shown to improve airway mechanics. This is the case of physical exercise where the increase in ventilation is associated with a parallel increase in partial forced expiratory flow so that work load at peak exercise can be achieved at near normal values.[58,59] Independently of the underlying mechanisms that are discussed in the next session, this observation implicates ASM dysfunction as a major determinant of the disease because if it were indeed inflammation or airway remodelling making airway narrowing fixed and limiting the effects of the DB in asthma, then exercise hyperventilation should be ineffective in improving airway function. Further support for this line of reasoning comes from a recent study examining the response to inhaled MCh in healthy subjects at high altitude.[53] Despite the occurrence of pulmonary suboedema as suggested by an increase in dynamic elastance and reactance at 5 Hz, airway responsiveness (AR) was decreased compared with sea level, and this was related to the increase in minute ventilation. The authors postulated that ventilation strongly opposes ASM shortening even in the presence of other mechanisms favouring narrowing.

Airway mechanics and lung elastic load. Ding *et al.* were the first to report that decreasing or increasing FRC by 0.5 L was associated with enhanced or blunted airway response to the active agent, respectively.[60] This provided the first evidence that lung volume is a major determinant of AR. Further studies conducted in humans under conditions of reduced FRC have strengthened this idea. For instance, in healthy humans shifting from seated to supine posture[61] or decreasing lung volumes by way of chest wall strapping[36,55] has been shown to cause larger decrements in airway size upon exposure to MCh, as suggested by an increase in lung or respiratory resistance, and also greater changes in dynamic elastance or reactance at 5 Hz, thus suggesting the occurrence of heterogeneous bronchoconstriction.

In rabbits, Xue *et al.*[62] reported that administering a positive end-expiratory pressure of 6 cm H_2O for a few days resulted in a significant decrease in AR. A study in mild-to-moderate asthmatics treated with continuous positive airway pressure (CPAP) of 8–10 cm H_2O for seven nights caused a significant improvement in AR to MCh.[63] According to Xue *et al.*,[64] the effects of CPAP could be due to remodelling of either the cytoskeletal organization of ASM or its contractile machinery by chronic strain or the non-contractile airway wall tissues.

ASM contraction models and AHR. The studies summarized above suggest that the ASM is a key determinant of AHR independently of its active or effecter role. In addition, they demonstrate that the contractile status of ASM is dynamically regulated by ventilation and lung volumes. Breathing fluctuations and lung inflation impose a load to the ASM (mechanotransduction) such that length and force are constantly controlled. As ASM is the major determinant of airway size, this suggests that ventilation and lung volume contribute to AHR in asthma. Two models have been proposed to explain the force–length relationship of ASM under dynamic conditions. First, upon stimulation, ASM shortens because of formation of two types of cycling cross bridges between actin and myosin. Rapid cross bridges are quite unstable and easy to disrupt with breathing. In contrast, latch bridges attach and detach more slowly, thus causing force maintenance. Achieving a static equilibrium will lead the airways to become too stiff, and thus unresponsive to the inspiratory load.[15,16] The causes of the unbalance towards the latch state condition remain unknown. However, factors within or outside the airways such as increased ASM mass, increased velocity of shortening, fixed airway remodelling, loss of interdependence between airways and lung parenchyma, reduced lung recoil and impaired depth of breathing have the potential to contribute to the reduced airway response to DB or lung hyperinflation. Second, the contractile apparatus exhibits plastic adaptation to different lengths.[65] For instance, increasing length of either stimulated or unstimulated ASM causes an immediate decrease in force that is then followed by gradual recovery.[66] In tracheal ASM strips of asthmatic subjects, length oscillation produced less force decrease and faster recovery than in healthy subjects.[67]

Airway wall stiffness in vivo *in asthma*

In asthma patients, airway wall thickness and stiffness are increased compared with controls.[68–70] Both features contribute to AHR. According to modelling studies, for a given degree of ASM shortening, an increase in thickness is associated with a decrease in airway calibre,[12] increase in airflow resistance[71] and airway closure in response to constrictor stimuli.[21] Moreover, an increase in stiffness reduces airway distensibility, thus limiting the benefits of both pharmacological treatment and ventilation.[72] Increased airway wall stiffness may also paradoxically modulate in part AHR in asthma as a result of an increased internal load.[73]

Airway stiffness can be measured by plotting airway conductance measured with the forced oscillation technique (FOT) and lung volume during a slow vital capacity manoeuvre (Fig. 3).[74] This study showed that at any lung volumes, the slope of the relationship was lower in a group of asthmatics than in normal controls, thus suggesting increased airway wall stiffness. Further, after inhaling a bronchodilator agent, the slope measured at RV and FRC significantly improved, thus suggesting that ASM contraction contributed to stiffen the airways within and below tidal breathing range. At total lung capacity, by contrast, the slope did not change with the medication, thus suggesting that stiffness was due to fixed remodelling. These findings open new horizons in the evaluation of the mechanisms of airway wall stiffness is asthma and potential treatment.

Spatial and temporal heterogeneities

Healthy lungs are heterogeneous in nature. Yet they empty quite homogeneously during forced expiration,[75] presumably as a result of flow interdependence mechanisms capable of minimizing the effects of parallel heterogeneity.

Lung heterogeneities have regained attention recently despite having been reported over 60 years ago in asthma studies.[11] Recent imaging studies have revealed that, during a bronchospastic attack, large ventilation defects occur across the lungs. Interestingly, nearby areas become paradoxically hyperventilated. This pattern may follow an avalanche-like behaviour and cause severe airflow obstruction and gas exchange impairment.[76] Mathematical integrative models provide an explanation of how lung heterogeneities form and progress over time. One hypothesis is that parallel heterogeneities occur at the periphery of the lung,[76,77] where tidal volume and intrabronchial pressure control airway dynamics. With ASM activation above a given threshold, small differences occur between two bifurcating airways. The lung region fed by the airway that narrows slightly more than the other one will become hypoventilated compared with before narrowing. As a result of the decreased physical stretch applied by this region to the wall, this airway will further narrow until ASM shortening is contrasted by local ventilation. Redistribution of part of the tidal volume to the other airway will result in

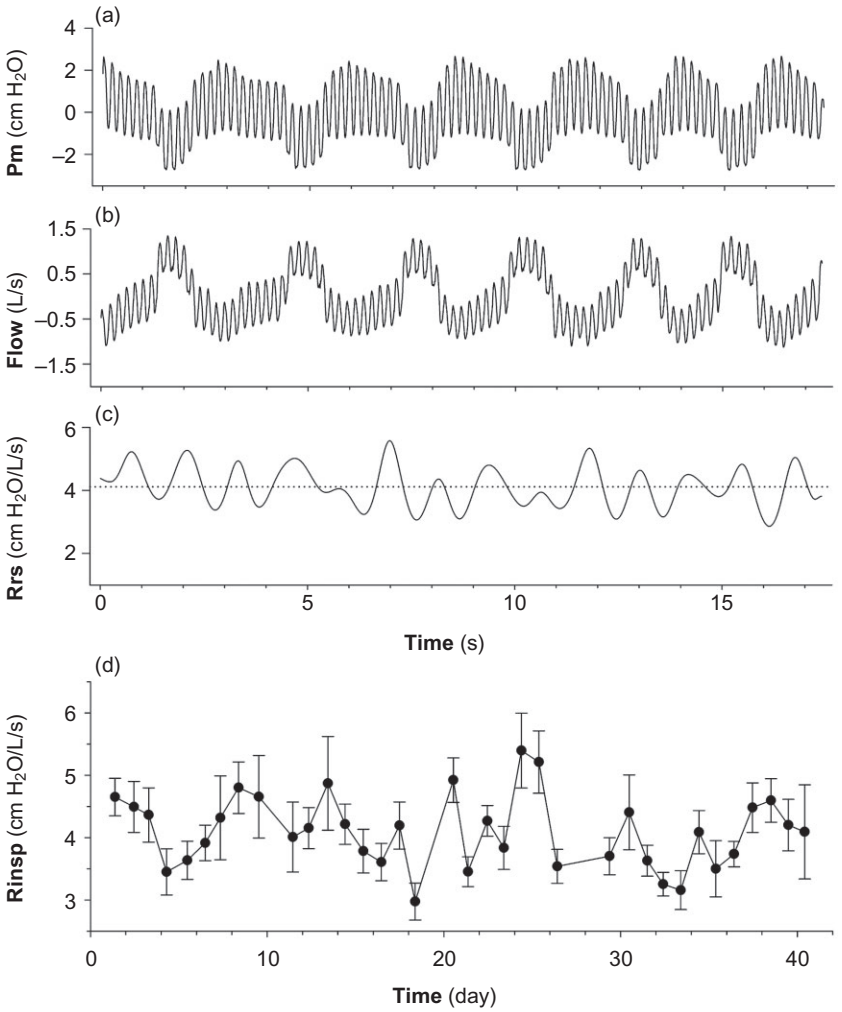

Figure 3 Forced oscillation technique (FOT) recordings in an asthmatic subject. Upper panel: pressure (Pm) and flow recorded at the mouth during forced oscillation at 5 Hz imposed on tidal breathing. Within-breath respiratory resistance (Rrs) is displayed below. Lower panel: average (solid circles) with standard deviation (bars) Rrs values (Rinsp) recorded during morning and evening sessions of 2 min each over 40 consecutive days. Note the high variability of Rinsp between days suggesting abnormal fluctuation of bronchial tone over time. Courtesy of Alessandro Gobbi and Raffaele Dellacà.

bronchodilatation. Replication of these phenomena on a larger scale will increase number and size of hypoventilated regions, thus precipitating the bronchospastic event. Presumably, this is not the only mechanism contributing to the formation of patchy hypoventilated lung regions in asthma. Serial heterogeneities of airway narrowing also contribute to direct ventilation to preferential lung regions, thus exaggerating the effects of parallel heterogeneities at a more peripheral level.[78]

Can the DB interrupt the gradual replication of the hypoventilated areas in asthma, thus limiting the severity of narrowing? Lavoie *et al.* provide evidence that this might not be the case for two major reasons.[79] Firstly, in individual airways, the effects of DB depend on the severity of narrowing. In the model of heterogeneous distribution of airway narrowing, it can be envisaged that, within lung regions served by unconstricted airways, the effect of the DB on airway size will be much greater than with the regions with constricted airways. Therefore, the DB cannot help but increase the ventilation heterogeneities, with the airways more and more constricted and unable to react to lung inflations. Secondly, as the dilator effects of the DB also depend on the depth of the breaths,[80] worsening of the constrictor conditions will lead to fatigue of the inspiratory muscles. In turn, this will reduce the strain imposed by ventilation on the airways, thus contributing to precipitate the attack.

There are several non-invasive methods for assessing ventilation distribution in clinical practice. The single-breath nitrogen washout is based on the analysis of nitrogen concentration plotted against expired volume.[11] Phase III is the alveolar plateau and is almost flat in healthy subjects as lungs empty homogeneously. In contrast, in lung obstruction, nitrogen concentration keeps on increasing with expiration because of lung regions with high time constants emptying late on expiration. A further increase in nitrogen concentration very late on expiration is designated phase IV and taken as a marker of airway closure. Interpretation of the results is not unambiguous in that phase III depends on both parallel and serial heterogeneities and closing volume on airway size and transmural pressure. In addition, the test variably interferes with bronchial tone and thus ventilation distribution because of the deep inspiration preceding the test. More recently, the multiple breath nitrogen washout (MBNWO) has been introduced to examine the ventilation heterogeneities at the acinar

and conductive airways levels.[81] The test is based on the determination of the slopes of nitrogen concentration versus volume during a series of tidal breaths while breathing 100% oxygen. In normoventilated lungs, these do not vary during breaths as all lung regions empty uniformly over time. In contrast, in heterogeneous lungs, different time constants cause a gradual increase in the slopes with tidal breaths, thus allowing conductive and acinar heterogeneities to be estimated.[81] Interestingly, both heterogeneities had been reported in asthma[82] and found to be related to the severity of AHR.[83] Further, ventilation distribution through the lungs can be assessed by the frequency dependence of respiratory impedance measured at the mouth.[84] In healthy lungs, increasing the oscillation frequency leaves airflow resistance relatively unaltered as it distributes quite homogeneously across the entire bronchial tree. In heterogeneous lungs in contrast, increasing the oscillatory frequency is associated with a decreased impedance as the oscillation signal preferentially distributes to the more ventilated lung regions, thus avoiding the regions with high flow resistance. This has been confirmed by comparing measured lung mechanics to mathematical models[38] and recently to imaging findings.[85]

Complex systems are not characterized only by processes involving a large range of structural and topographical scales. Time scale is another crucial dimension of asthma. The field owes Dr Peter Macklem credit for examining the temporal fluctuation of lung function in asthma as a function of the intrinsic mechanisms of bronchospasm.[7] He measured input impedance (Zrs) over a period of 15 min in a group of healthy individuals in seated and supine positions. Measurements were repeated after a dose of inhaled MCh. The data were contrasted with a group of asthmatics. Interestingly, in the healthy subjects exposed to MCh in either postures, lnZrs reached values similar to the asthmatics'. Standard deviation of lnZrs became indistinguishable between groups when supine posture and MCh were combined. Variability and mean Zrs values were not related to each other. Dr Macklem interpreted the findings as ASM activation, unloading and decreased lung stretch representing the key mechanisms controlling bronchial tone. As often occurs in research, the findings were not fully reproduced in following studies.[4,36] However, this study represented a breakthrough and milestone in respiratory medicine because, for the first time, the disease was examined in its temporal domain. Since then, many studies have tested the hypothesis that if exaggerated temporal variability of bronchial tone is the typical expression of the disease, then this could predict the occurrence of exacerbations, the effects of therapy and symptoms. By examining PEF time series for a duration of 6 months, in 80 asthmatics treated with placebo, salmeterol and salbutamol, Frey *et al.* were able to predict the risk of asthma attacks 1 month in advance.[5] Thamrin *et al.* predicted the clinical worsening in 87 asthmatics after withdrawal of inhaled steroid therapy on the basis of the PEF coefficient of variation.[8] Our group measured airflow resistance at 5 Hz (R5) for 2 min of tidal breathing twice a day for 6 months in asthmatic and healthy subjects.[6] Temporal fluctuations of airway calibre estimated from the coefficient of variation of R5 (R5CV) were significantly larger in the asthmatic than in the healthy subjects at any time scales between 1 and 32 days. In addition, R5CV measured over an 8-day period predicted the probability of an acute episode of airway narrowing within the next 4–30 days.

Taken together, these studies suggest that differences between short- and long-term fluctuation patterns might be explained in the light of fractal behaviour of bronchial tone, with short-time series exhibiting significant results only if exposure to asthmogenic triggers is sufficiently intense to render airway mechanics unstable between breaths.

New data further support Dr Macklem's idea that short-term variability of R5 is a reflection of ASM activity. In a recent study, we exposed healthy and asthmatic subjects to MCh and showed that the interquartile range of the frequency distribution of R5 was significantly related to the velocity of renarrowing after the DB.[36] If the latter is a determinant of the ASM velocity of shortening, then it is reasonable to think that this contributes to making the airways unstable even between tidal breaths.

NEW PERSPECTIVES OF LUNG FUNCTION IN ASTHMA

The perspective proposed in this review is founded on the following concepts. First, asthma is a complex disease because the lung is a complex organ. To cause an attack, the trigger has to interact with the lung at several levels, and the final outcome might be fairly independent of the intensity of the stimulus. Second, there is sufficient evidence that ASM abnormalities play a crucial role in causing bronchospasm, but this has to be considered in the light of airway wall inflammation and remodelling, and ventilation. Finally, further complexities result from the topographical distribution of the response across the lungs and the ability to control bronchial tone over time. With this in mind, we need to examine the full spectrum of mechanisms and interactions occurring in asthmatic patients and then focus on those through which bronchospasm most likely occurs. In our view, such investigations should be undertaken within the framework of the topographical, volume and time domains of the lung. New technologies such as FOT and MBNWO now provide the necessary tools to achieve this. FOT allows assessment of airway mechanics with great sensitivity and accuracy under any volume and time conditions, estimating ventilation heterogeneities and examining subjects of any age with minimal cooperation and without any invasiveness. In addition, standards have recently been issued, complete with predicted values,[84] and softwares are now available for immediate online results and/or subsequent analysis. The MBNWO is another non-invasive technique capable of exploring the disease with great accuracy in the peripheral part of the lung. Standards have recently been issued.[86] Combining the two tests may help address questions unsolvable until now

with relative simplicity and applicable to individual subjects for diagnosis and follow up, assessment of the site of airway narrowing, airway wall stiffness, effects of loading and DB with ensuing recovery and temporal fluctuation of bronchial tone. With this information in hand, therapy could really be tailored to individual patients with treatments directed against the precise mechanism underlying the decrease in airway calibre for each individual patient. These tools provide the field with the means with which to move forward and make rapid progress in understanding the pathogenesis of asthma.

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