Comprehensive Physiology

Asthma and lung mechanics

Journal:	*Comprehensive Physiology*
Manuscript ID	Draft
Wiley - Manuscript type:	Overview Article
Date Submitted by the Author:	n/a
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Keywords:	pulmonary mechanics < Respiratory Physiology, lung < Respiratory Physiology, asthma < Respiratory Physiology
Abstract:	This review will discuss in detail the pathophysiology of asthma from the point of view of lung mechanics. In particular, we will explain how asthma is more than just airflow limitation resulting from airway narrowing, but in fact involves multiple consequences of airway narrowing, including ventilation heterogeneity, airway closure, and airway hyperresponsiveness. In addition, the relationship between the airway and surrounding lung parenchyma is thought to be critically important in asthma, especially as related to the response to deep inhalation. Furthermore, dynamic changes in lung mechanics over time may yield important information about asthma stability, as well as potentially provide a window into future disease control. All of these features of mechanical properties of the lung in asthma will be explained by providing evidence from multiple investigative methods, including not only traditional pulmonary function testing, but also more sophisticated techniques such as forced oscillation, multiple breath nitrogen washout, and different imaging modalities. Throughout the paper we will link the lung mechanical features of asthma to clinical manifestations of asthma symptoms, severity and control.

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Running Head: Asthma and Lung Mechanics

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Didactic Synopsis

Major Teaching Points:

- The pathophysiology of asthma involves much more than just airway narrowing as seen by airflow obstruction on spirometry.
- The consequences of airway narrowing include increased airway resistance, airtrapping, dynamic hyperinflation, ventilation heterogeneity, airway closure and airways hyperresponsiveness

- Airway-parenchymal interdependence plays an important role in determining lung mechanics in asthma. In particular, interdependence influences airway smooth muscle constriction and the response of the lung to deep inhalation.
- Changes in the physical properties of the lung parenchyma are also involved in the pathophysiology of asthma, and include changes in lung structure and surfactant that may result in altered lung compliance.
- Multiple modalities may be used to detect changes in lung mechanics in asthma, including spirometry, changes in lung volumes, forced oscillation technique, single and multiple breath nitrogen washout, and various techniques of lung imaging such as CT, SPECT, PET, and He3-MRI.
- The clinical manifestations of asthma reflect multiple aspects of altered lung mechanics.

Introduction

Asthma is a complex lung disease that traditionally has been defined as variable and excessive narrowing of the airways in response to a variety of stimuli, which leads to airflow limitation and the clinical manifestations of cough, wheezing, shortness of breath and chest tightness. Symptoms are highly variable in their expression, occurring at rest, or only upon exposure to an irritant or other trigger, like air pollution, allergen, upper respiratory infection, or exercise. Comorbidities also contribute to the severity and control of symptoms, such as obesity, gastroesophageal reflux disease (GERD), rhinosinusitis, and emotional stress. The diagnosis is made by demonstrating periodic airflow limitation by spirometry, or airflow limitation that is alleviated by bronchodilator, in the context of the clinical presentation. Airway hyperresponsiveness (AHR) may also be demonstrated by direct or indirect bronchial challenge testing in order to confirm a diagnosis.

However, asthma involves much more than simple airway narrowing. Indeed, over the past few decades, multiple observations and experiments have revealed the complexity of lung mechanical abnormalities that occur in asthma, which culminate in airway narrowing and AHR, the hallmarks of asthma [12, 228]. Among these are the behavior of airway smooth muscle, the importance of interdependence between lung parenchyma and airways, the effects of deep inflation, the role of ventilation heterogeneity and airway closure, and mechanical changes in the lung parenchyma. These abnormalities of lung function in asthma are thought to contribute to the clinical expression of the disease related to asthma severity and control. In this review, we

will provide detailed descriptions of these various abnormalities of lung mechanics in asthma, and relate them to how they are expressed clinically in patients with asthma.

The Pathology of Asthma

Traditionally asthma has been considered a disease of airway inflammation. The old dichotomy of intrinsic vs. extrinsic asthma, which was thought to relate to non-allergic vs. allergic asthma, respectively, has been replaced with a number of different endotypes and phenotypes that reflect the underlying pathology and clinical expression of the disease [201]. A more contemporary view has been that asthma is driven by eosinophilic or non-eosinophilic inflammation, with the former categorized as under the control of T-helper type II cell regulation (TH2) [54, 195]. (Figure 1). In particular, upon exposure to allergens, TH2 cells promote the release of cytokines such as IL-4, IL-5 and IL-13 that result in eosinophil recruitment and activation in the airways, elaboration of IgE and activation of mast cells, and stimulation of airway smooth muscle and mucus glands. In addition, upon exposure to viruses and airway irritants, non-allergic TH2 stimulation may lead to IL-5 and IL-13 release from innate lymphoid type 2 cells and resultant eosinophilic inflammation. In contrast, other patients with asthma may display a non-TH2 type inflammatory response characterized by neutrophilic inflammation, typically with the involvement of TNF-alpha [106], and TH-17 cells and the elaboration of IL-17A, IL-17F and IL-22 [9]. Patients with severe asthma may have high levels of IFN-χ in their airways, suggesting the role of infection in driving disease [219]. The resulting inflammation of the airway results in airway wall edema, inflammatory cellular infiltration, and mucus hypersecretion. There is also reticular basement membrane thickening with collagen deposition in the subepithelial space.

Deposition of proteoglycans and glycosaminoglycans is also found in this region of the airway wall, and has been correlated with the severity of asthma, decline in FEV1 [105] and airway hyperresponsiveness [107]. The consequence of these inflammatory events is airway luminal narrowing, with resultant airflow limitation. Ongoing inflammation and injury to the airway epithelium may lead to activation of the epithelial/mesenchymal trophic unit, resulting in remodeling of the airway [30]. Airway remodeling involves increased vascularity, subepithelial myofibroblasts, fibrocytes, goblet cell hyperplasia, and increased airway smooth muscle mass [195]. Mechanical stress of airway epithelial cells in vitro has also been shown to elicit a remodeling response in tissue fibroblasts, which suggests that alterations in mechanical stress in the lung can augment matrix remodeling of the airway [246]. Such changes would be expected to stiffen the airway, and indeed decreased airway distensibility has been shown in some [35, 116, 129, 280, 287], but not all [284] studies of patients with asthma. In addition, mechanical compression of airway epithelium appears to contribute to ASM proliferation and contractility [149]. Recently, another type of abnormal airway epithelial cell behavior has been observed, known as jamming. In vitro, healthy epithelial cells are very mobile and fluid-like, but airway epithelial cells from patients with asthma appear less motile and jammed in place. The clinical significance of this behavior is unclear, but it appears to be modulated by mechanical stress such as might occur during bronchoconstriction [197, 199].

The inflammation involved in asthma extends from the distal airways to the lung parenchyma [92, 143]. This inflammation differs between patients with controlled vs. non-controlled asthma with uncontrolled asthma involving more myofibroblasts and increased percentage of collagen, versican and decorin in the lung parenchyma [282]. Other studies have shown increased

myofibroblasts in both fatal and non-fatal asthma compared to healthy controls [18], and a wide distribution of eosinophils throughout the entire airway tree extending to the peribronchial parenchyma in fatal asthma, along with mast cells and neutrophils in this region [19]. A disrupted elastic fiber network of the lung parenchyma in patients with fatal asthma, along with peribronchial inflammation surrounding the small airways, may lead to loss of functional mechanical linkage between airway and surrounding parenchyma, leading to effective unloading of airway smooth muscle and nearly unopposed bronchoconstriction [170]. In addition, severe asthma has been associated with loss of elastic recoil of lung that appears to be due to microscopic emphysema that is not evident on pulmonary function testing (i.e., by the diffusing capacity of the lung for carbon monoxide test (DLCO)) or by chest CT imaging [80].

Despite the solid evidence of airway and parenchymal inflammation in asthma, there are poor relationships between airway inflammation and asthma severity [281] or AHR [4, 38]. This fact emphasizes the complex nature of the clinical expression of asthma, which cannot be fully explained by structural abnormalities and inflammation alone. Additional factors, to be reviewed in this paper, include more subtle manifestations of abnormalities in lung mechanics, as well as the emergence of other lung properties that characterize this complex disease.

Airway Narrowing in Asthma

Airway Narrowing and Resistance to Airflow

The fundamental problem in asthma is excessive narrowing of the airway. Based on Ohm's Law, the resistance to airflow is equal to the driving pressure divided by the flow. When flow is laminar, we can apply Pouseille's Law to describe the relationship between flow ($\dot{V}$), pressure (P) and resistance as they relate to the length (L) and radius (r) of the conduit, as well as the density and viscosity of the gas (Equation 1):

$$\Delta P = 8\mu L\dot{V}/\pi r^4 \qquad \text{Equation 1}$$

The critical role of airway diameter in determining the resistance to flow is seen easily by the 4th power inverse relationship of airway radius to airway resistance. Thus, small changes in airway diameter result in large changes in airway resistance. These changes subsequently result in multiple manifestations of abnormal lung mechanics (Table 1).

Airway narrowing results in wheezing and increased resistive work of breathing. Wheezing results from the high frequency vibration of the airway wall as it oscillates between narrow and less narrow diameter within a region of airway narrowing that is so severe that the airway walls nearly touch each other. In such a region, the velocity of gas flow increases, resulting in a drop in intraluminal pressure due to Bernoulli's principle, which results in further airway narrowing. Meanwhile, the ongoing flow of air eventually builds up enough pressure to push the airway wall apart again, and the cycle repeats, resulting in a high frequency vibration of the airway wall around 400 Hz and the clinical sound described as a wheeze [101].

Airway narrowing results in expiratory airflow limitation [204]. The mechanism of flow limitation is usually described based on the development of an equal pressure point within the airway along the path of flow from alveoli to mouth [174]. Because the airways are compressible, the falling pressure gradient from alveoli to mouth results in a point along the airway where inside pressure equals outside pressure surrounding the airway. From this point on distally toward the mouth, the outside pressure now exceeds the inside pressure, and the airway narrows. Due to Bernoulli's principle, gas velocity must increase within the narrowed airway segment, which results in a drop in pressure within the segment in order to conserve net energy. This drop in pressure results in further airway narrowing. Thus, a cycle is established of airway narrowing during exhalation, which limits flow. Another mechanism of flow limitation that occurs is due to wave speed theory [60]. This theory states that gas flow cannot exceed the speed with which the pressure perturbation travels within the walls of a compressible airway. Maximal flow is then governed by airway diameter, airway wall compliance and gas properties, all of which relate to maximal flow described by wave speed theory.

Expiratory flow limitation may occur not only during forced exhalation, but also at rest. Indeed, expiratory flow limitation during tidal breathing may occur in patients with asthma and COPD and even otherwise healthy people with obesity [165], contributing to shortness of breath among these groups.

Airway narrowing not only results in flow limitation and wheezing, but also increased work of breathing. The work of breathing is due to the increased pressure required to move air through narrowed regions, as well as the development of unequal time constants throughout the lung.

The time constant is the product of resistance (R) and compliance (C), and relates to the time necessary to fill or empty the region in question. With increased R, it takes more time for air to flow into or out of the airway segment. With increased C, it takes more time to fill or empty the lung volume served by the airway segment. Unequal time constants result in decreased dynamic compliance, especially with increasing breathing frequency (frequency dependence of compliance) [87, 294]. This translates functionally into increased work of breathing.

Airway narrowing with resultant prolonged time constants throughout the lung also results in gas trapping at rest, and dynamic hyperinflation with activity. The latter is especially significant because it is the most important factor contributing to dyspnea on exertion [155] and impaired activities of daily living [261]. With increased air trapping over time, the diaphragm becomes more inefficient at contraction, and increased intrathoracic pressures may also reduce preload and cardiac output. Interestingly, dynamic hyperinflation in response to methacholine has been shown to occur without expiratory flow limitation [248], and may be related to persistent activation of inspiratory muscles, raising FRC perhaps to reduce airway resistance, expiratory flow limitation and/or airway closure [194, 209].

Airway narrowing is typically uneven in nature, leading to uneven distribution of time constants throughout the lung and ventilation heterogeneity, which is a key component of abnormal lung mechanics in asthma. Ventilation heterogeneity will be discussed in more detail below.

Detection of Airway Narrowing

There are multiple ways to measure airway narrowing or its consequences.

Spirometry

Airway narrowing is usually inferred from spirometry, where the reduction in airflow results in a decrease in the ratio of FEV1/FVC. This empiric measure was first described by Tiffaneau and is sometimes referred to as the Tiffaneau index. Because it is measured during a forced exhalation, maximal airflow becomes effort independent once about 20% of the vital capacity has been exhaled due to the development of airway narrowing and flow limitation. The FEV1 occurs well within the effort independent portion of the flow-volume relationship, thus rendering it especially robust as a measure of airway function. While the GOLD guidelines define the normal range of FEV1/FVC as > 0.7 (https://goldcopd.org/), the ATS/ERS have defined the statistical 5th percentile of the distribution of values in a normal healthy population as the lower limit of normal [58]. Importantly, the FEV1/FVC ratio declines with age, so the concept of using the lower limit of normal is more realistic. Another way to describe the lower limit of normal is to equate the 5th percentile with the number of standard deviations below the mean, which is 1.64 SD [217]. This number is called the z-score. Thus, the LLN of the FEV1/FVC ratio is a z-score of -1.64. Classically, the FEV1 and the ratio of FEV1/FVC have been thought to reflect airway narrowing, and, in particular, large airway (> 2mm diameter) narrowing. This seems to have been confirmed by Brown and colleagues, who demonstrated that only changes in large airway dimensions by CT scan correlated with changes in FEV1/FVC and FVC [37]. Meanwhile, other studies involving functional imaging have shown that large airway constriction alone cannot account for changes in ventilation seen after bronchoconstriction, and some degree

of small airways involvement is necessary [250]. These types of studies trying to correlate structre and function all have intrinsic limitations that makes generalizing their results difficult, so it is not entirely clear which airways are involved in changes in FEV1 and FVC.

Airway Resistance

Airway narrowing may also be inferred from direct measures of airway resistance [120]. Airway resistance (Raw) is usually measured clinically by body plethysmography, but may also be measured by other techniques including forced oscillation and interrupter. During body plethysmography, mouth pressure taken at a moment of no flow through the airway is measured as a surrogate for alveolar pressure, and flow through the airway is also measured. Airway resistance is thus calculated as alveolar pressure divided by flow. Since Raw is highly dependent on lung volume, Raw is more accurately expressed as specific Raw (sRaw), the product of Raw and the volume at which the measurement is made (thoracic gas volume, TGV), or specific airway conductance (sGaw), which is the reciprocal of Raw, Gaw, divided by TGV.

Airway resistance can also be measured by the forced oscillation technique (FOT), during which an oscillating flow of gas of different amplitudes and frequencies is delivered at the mouth during spontaneous breathing [14]. The resulting pressures measured encompass the entire impedance of the respiratory system (Zrs), which is composed of real and imaginary components. The real component, which is that part of the pressure signal in phase with flow, reflects Newtonian resistance at higher frequencies (i.e. > 5Hz), and thus is a direct measure of overall airway resistance. At frequencies below 5 Hz, resistance rises with decreasing frequency,

displaying frequency dependence of resistance, which is thought to reflect airflow heterogeneity, tissue viscoelasticity and airway wall shunting. The imaginary component is that part of the pressure signal out of phase with flow and is known as reactance, which itself is composed of both elastance and inertance.

Airway resistance may also be measured by the interrupter technique [142]. During this procedure, a patient breathes quietly through a mouthpiece and the flow of air is briefly interrupted by a shutter occlusion. Pressure is measured at this point, and is divided by the flow immediately prior to flow interruption according to Ohm's Law to measure resistance. This technique provides a measure of respiratory system resistance, but the resistance specifically attributable to gas flow in the airways cannot be determined .

All of the above methods provide a measurement of overall airway resistance across the airway tree, with the caveats stated. To measure airway resistance more specifically in the lung periphery, a more invasive measurement of airway resistance can be performed by inserting a catheter into a small airway to directly measure pressure and flow. Using this method, Ohrui and colleagues demonstrated that the peripheral airways were hyperresponsive to methacholine [193]. A similar approach is by the wedged bronchoscope technique, in which a steady flow of gas is instilled directly into a wedged airway, generating a resultant pressure due to the resistance to outflow of gas via the collateral airways that serve the wedged segment [275]. The relationship of pressure divided by flow is the overall resistance in the peripheral airways that conduct collateral flow out of the segment. Since these peripheral airways are thought to be the respiratory bronchioles, this technique measures resistance in these small, peripheral airways.

This technique has shown that peripheral airway resistance is elevated in asthma, even in those patients with normal spirometry, and that the peripheral airways are hyperresponsive to stimuli such as histamine [273], cool dry air [124], and bradykinin [19].

Imaging

Airway narrowing may also be assessed directly by imaging using CT. FEV1 is correlated with airway wall measurements of the more distal 4th to 6th generation of airways [104]. Airway narrowing has been found to be more heterogeneous in asthmatic than in healthy individuals [135]. Optical coherence tomography (OCT) is a relatively new technique that involves imaging the airway wall at the time of bronchoscopy and has yielded insight into airway wall structure and mechanical properties related to airway narrowing [284].

Lung Volumes

As discussed, the consequences of airway narrowing are airflow limitation, seen by spirometry, and increased airway resistance, measured by various techniques. The important consequence of gas trapping is usually detected by measuring lung volumes, in particular the ratio of RV/TLC. Dynamic hyperinflation is detected by rising end-expiratory lung volume (EELV) during exercise, which may be measured indirectly by the inspiratory capacity [41]. With increasing dynamic hyperinflation, EELV rises, resulting in a fall in IC. Air trapping is seen visually by CT scanning that quantifies air trapping by CT density <850 HU at FRC [40, 43].

Mechanisms of Airway Narrowing in Asthma

The traditional concept of airway narrowing is based on two main mechanisms, one relating to bronchoconstriction from activation and shortening of airway smooth muscle (ASM), and the other related to luminal narrowing from surrounding airway wall thickening from edema and inflammation. However, we now understand that additional mechanisms are also involved [97] (Figure 2).

Airway Smooth Muscle Dynamics

There is no doubt that ASM contraction results in airway narrowing by mechanical constriction of the airway [4]. The importance of this mechanism is easily realized by the fundamental clinical success of treating airway narrowing with beta agonists that relax ASM and allow bronchodilation. Airway smooth muscle is arranged concentrically around the airway wall, but also spirals in a longitudinal direction, such that activation of ASM results in both narrowing and shortening of the airway. For many years there has been controversy about whether there is increased mass of ASM (hypertrophy, from increased cell size, and hyperplasia from increased cell number), or whether there is simply increased activation or strength of ASM shortening. It now appears that both mechanisms are involved [4, 213]. In addition, the velocity of ASM shortening is increased in patients with asthma. Furthermore, increased basal tone in ASM is associated with increased AHR and the reduced ability to bronchodilate in response to a deep breath [77]. However, animal work has demonstrated that ASM dynamics at the cellular level

alone cannot fully explain airway narrowing, implicating important roles for the intact airway wall and surrounding structural environment [25, 150]. ASM function is also directly linked to airway inflammation and remodeling [188].

In addition, the load against which ASM must react also is an important determinant of airway narrowing. With decreased loads, ASM will constrict more for a given force of contraction. There are both internal and external loads to ASM [97, 127]. Internal load is determined by the structural elements of the airway wall, including the ASM itself, as well as the buckling of the basement membrane. External load is determined by the structural and functional forces that resist ASM constriction outside the airway wall. This linkage between airway and surrounding lung parenchyma is referred to as airway-parenchymal interdependence [196]. In asthma, external ASM loads are thought to be decreased because of peribronchial inflammation and edema, which serve to uncouple the airway wall from the surrounding alveolar tethering units, a phenomenon referred to as loss of interdependence. Loss of interdependence not only allows the ASM to constrict more for a given force, but also uncouples the airway wall from the lung parenchyma such that the airways dilate less in response to deep inhalation. With less bronchodilation from a deep breath, ASM is maintained in a more constricted state for longer periods of time, which may lead to further difficulty in stretching the ASM during a deep inhalation. Over time this may lead to remodeling of the ASM such that it is "frozen" in a "latch state" of actin-myosin interaction that hinders relaxation [4].

Airway Inflammation and Edema

Inflammation and edema of the airway wall will increase the thickness of the wall. The consequence of this is that for a given degree of ASM constriction, the luminal diameter will decrease more due to geometric factors [15]. Inflammation and edema also contribute to altered contractility of ASM. Inflammation and edema of the airway wall and peribronchial region are thought to be the main reason for loss of airway-parenchymal interdependence [213] (see below).

Airway Remodeling

The combined influences of changes in ASM, airway inflammation and edema, as well as changes in other airway wall components, including the epithelium, mucus glands, basement membrane, and blood vessels, all contribute to airway remodeling [71]. Such remodeling appears to have an additive effect on airway narrowing when in the presence of active inflammation [278]. Airway remodeling contributes to airway narrowing by not only increasing airway wall thickness, but also changing airway wall compliance [138]. Indeed, in asthma there is less distensibility of airway walls, which is thought to be due to remodeling [35, 131]. This reduced distensibility allows less dilation in response to a deep inhalation, even in the presence of normal airway – parenchyma interdependence. Reduced dilation in response to a deep inhalation to revere bronchoconstriction may also be due to more rapid constriction during exhalation [110]. Interestingly, increased stiffness of the airway wall may also be protective against bronchoconstriction [31]. In obesity, patients with asthma may have more compliant airway walls, leading to more severe airway narrowing and either actual or functional (i.e., very prolonged time constants) closure [1]. There is clinical evidence that repeated

bronchoconstriction, in the absence of airway inflammation, leads to airway remodeling [86], but this finding has not been replicated in experimental animals [168].

Loss of Airway-Parenchymal Interdependence

As mentioned previously, loss of interdependence may unload ASM and lead to more severe airway narrowing for a given force of contraction, as well as failure to dilate in response to a deep inhalation. The failure of patients with asthma to bronchodilate in response to a deep breath has been measured most directly by noting changes in Raw before and after a deep breath [182]. In 1981, Fish suggested that the failure to bronchodilate in response to a deep breath may contribute to AHR [72]. The response to a deep breath depends not only the linkage of parenchyma to airway wall and the distensibility of the airway, but also on the speed of airway re-narrowing [113, 208, 224].

The response to deep breath has also been inferred by noting changes in airflow at a given lung volume when comparing flows measured following a deep (maximal, M) inhalation, compared to flows measured following a submaximal (partial, P) inhalation, which avoids the full, deep breath involved in conventional spirometry. When plotted on the same volume axis, the ratio of flow after a deep breath vs. after a partial breath is greater than one (M:P > 1) if bronchodilation occurs, and is less than one (M:P < 1) if bronchoconstriction occurs.

One explanation given for the response of the airway to deep inhalation is related to whether or not the ASM relaxes or perhaps even constricts following a deep breath. A relaxation response

may be due to production or release of nitric oxide by ASM [227] or increased surfactant response to DI [45]. Another explanation is more complicated and relates to the ratio of airway to parenchymal hysteresis [76]. Hysteresis refers to the difference in the pressure vs. volume relationship observed when one inhales vs. exhales. If the lung were a perfect elastic substance, the PV relationship during inhalation would be the same as that during exhalation. However, as a viscoelastic material, together with the properties of surfactant, the PV curve on inhalation demonstrates lower compliance than that on exhalation, and generally forms a loop.

The airway wall can be considered to have similar material properties. The hysteresis of each structure can be thought of as reflecting the elastic recoil of that structure [207]. Thus, if the hysteresis of both airway and lung were the same, then there would be no response to a deep breath, and M:P = 1, because the inward airway recoil would balance the outward parenchymal recoil. If airway hysteresis exceeded parenchymal hysteresis, then the airway would be at a larger diameter after a deep breath than before, a bronchodilator response with an M:P ratio > 1, because airway recoil in would lag behind the restoration of parenchyma recoil out; i.e., the response would be dominated by the outward parenchymal force. If airway hysteresis were less than parenchymal hysteresis, then the airway diameter would be smaller after a deep breath than before, a bronchoconstrictor response with an M:P ratio < 1, because parenchymal recoil out would lag behind airway recoil in; i.e., the response would be dominated by the inward recoil of the airways (Figure 3).

Healthy subjects and those with mild asthma typically bronchodilate in response to a deep breath, with M:P > 1. Patients with moderate to severe asthma either don't bronchodilate, or

may even constrict after a deep breath (M:P < 1). In the latter case, inflammation of the airway wall and surrounding parenchyma is thought to result in a situation where there is both uncoupling of the airway from the parenchyma, thus not allowing the parenchyma to pull on and tether open the airways, as well as increased hysteresis of the parenchyma. This latter effect creates a situation such that after the deep breath, the ASM constricts to a greater extent before it is sufficiently opposed by the outward force of the surrounding lung parenchyma, thus allowing a net bronchoconstriction to occur.

These responses to deep inhalation have consequences for patients with asthma [46]. First, they may not be able to relieve bronchoconstriction by a deep inhalation, and this lack of response to deep inhalation may, likewise, not protect them from bronchoconstriction. This bronchoprotective effect of DI in healthy subjects is lost in patients with asthma [128]. Studies in healthy subjects suggests that the bronchoprotective effect of DI is related to protection against airway closure rather than a direct effect on airway narrowing [45]. Second, the latch state of ASM may develop from inability to stretch ASM over periods of time, although this is only inferred from in vitro data [74, 132, 144]. A third consequence of loss of interdependence is potential risk of sudden, catastrophic airway narrowing and closure [269]. This may be due to reduced ASM load and inability to bronchodilate with a deep inhalation. Indeed, patients with near fatal asthma have been shown to have increased lung compliance with no evidence of parenchymal emphysema by measurement of DLCO or by high resolution CT scan. On microscopy, such patients may have disruption of alveolar elastic fibers, which would otherwise serve to provide interdependence with the airway through a mechanical tethering effect [80]. With decreased interdependence, the airways would be expected to be more uncoupled from the

surrounding lung parenchyma, leading to increased bronchoconstriction for a given stimulus and higher risk of a severe asthma episode. Another possible consequence of loss of interdependence is related to the effect of DI in obese patients with asthma. Specifically, the effects of a DI in obesity appear to depend on whether the DI is performed before or after methacholine challenge. If performed before, the DI enhances the response, whereas if performed after methacholine challenge, DI protects against the response [235]. The mechanisms involved in this differential response and how it relates to obesity are unknown, although it is speculated that the DI before challenge leads to opening of peripheral airways that then receive more methacholine than they would have otherwise, leading to enhanced bronchoconstriction, whereas the DI after challenge is able to relieve bronchoconstriction.

Dynamic Changes in Airway Narrowing over Time

Airway narrowing is not a static phenomenon. In fact, asthma is clinically defined by variable airflow limitation, which is typically relieved by bronchodilator, and AHR, the enhanced propensity and response to bronchoconstriction after exposure to triggering stimuli. This can make a diagnosis of asthma challenging because any one measurement of airway function at one point in time may or may not reveal any abnormalities. Accordingly, to help in making a diagnosis of asthma, the airways are typically challenged with bronchodilator, or likewise with bronchoconstrictor. One may also observe the dynamic nature of airway narrowing in patients with asthma during exercise. Increased physical activity results in increased metabolic and hence ventilatory demands. The latter are met by increasing both rate and volume of breathing. With airway narrowing and unequal time constants throughout the lung, some regions will

require more time to fill and empty completely, and such time will become more and more limited as the respiratory rate increases. The result may be a rise in EELV from insufficient time for exhalation, a phenomenon known as dynamic hyperinflation. Dyspnea becomes limiting during exercise when dynamic hyperinflation reaches a critical level and the inspiratory reserve volume falls to minimum [151]. Interestingly, there are data to also support another mechanism of hyperinflation, which may also occur with exercise, which is sustained inspiratory muscle activity [205]. Presumably this response helps maintain a higher EELV, which would result in reduced airway resistance from breathing at higher lung volumes. A recent study examined the relationship between changes in airway resistance, lung volume and airway diameter in the setting of dynamic hyperinflation and concluded that the increase in EELV was not passively related to increased airway resistance and reduced time for emptying, but rather was an active process, possibly due to increased inspiratory muscle activity, designed to increase EELV to maintain open the least stable airway, [194].

Variability in airway narrowing may also occur across different time scales. The FOT has been used to demonstrate that Zrs varies across minutes in asthmatic patients compared to healthy controls, presumably reflecting variability in the degree and distribution of airway narrowing. The complexity of lung impedance over the course of a 2 minute measurement also differs in asthma as measured by increased approximate entropy [84, 265]. Many studies have demonstrated variability in peak expiratory flow across hours during the day, and day to day over weeks and months. This variability can be quite striking, particularly in asthmatics with poor control of their disease, or in those with nocturnal symptoms [169]. In the latter case, PEF

variation occurs in wide swings according to what appear to be underlying circadian rhythms [169].

Recently, different groups have assessed the time course of PEF variability and found it to follow a power law, suggesting a fractal pattern over time that might be reminiscent of the fractal structural form of the airway tree (Figure 4). By applying a signal processing technique known as detrended fluctuation analysis, asthmatic patients may have predictable patterns of fluctuation that might provide a probabilistic estimate of future exacerbations [126, 251]. Other methods of quantifying variability of lung function over time, such as sample entropy [84], or fluctuation analysis with FOT [266], have shown that such variability is associated with asthma exacerbation frequency.

Airway Closure

At the extreme of airway narrowing is airway closure. Airway closure may describe the actual physical closure of airways, but also commonly reflects functional closure; i.e. such extreme narrowing of airways that airflow is essentially minimal and the airway behaves as if it is actually closed. Airway closure has been detected in asthma and is increasingly thought to play a significant role in the pathophysiology and clinical manifestations of the disease.

Detection of Airway Closure

Spirometry, Lung Volumes - FVC, RV/TLC

Airway closure is measured from spirometry as the forced vital capacity (FVC), representing the volume forcibly exhaled from maximal inspiration i.e. the volume between total lung capacity (TLC) and residual volume (RV). As such, the interpretation of FVC as a measure of airway closure is based upon the assumption that changes in FVC are due to changes in RV without a change in TLC. This appears to be a valid assumption in patients with asthma during indirect bronchial challenge, since methacholine causing a ~30% fall in forced expiratory volume in 1 second (FEV_1) did not alter TLC [140]. However, baseline TLC can be substantially increased in asthmatics at baseline and during an acute exacerbation so that the FVC greatly underestimates the increase in airway closure [37, 291].

In order to more reliably measure airway closure, direct measurement of TLC and RV is required. This is most commonly performed with either body plethysmography or inert gas dilution. Body plethysmography involves placing a patient inside an airtight box and having them breathe against a closed shutter. By measuring the changes in box pressure during inspiration and expiration and invoking Boyle's law (the product of pressure (P) and volume (V) under initial circumstances (P1, V1) will equal the product of P and V under another set of circumstances (P2,V2); i.e., $P_1V_1 = P_2V_2$), the lung volume at which panting occurred can be measured. Instructing the patient to then perform a full inspiratory and expiratory maneuver allows calculation of other lung volume subdivisions, including TLC and RV. Notably, since the body plethysmography technique assumes an equilibration of mouth and alveolar pressure during measurement of TGV, this method may overestimate TGV in patients with airflow limitation in whom such equilibration may not occur during the time period of measurement [230]. Another

technique to measure lung volumes is by the inert gas dilution method, which utilizes the principle of conservation of mass (the product of concentration (C) and volume (V) under initial circumstances (C1, V1) will equal the product of C and V under another set of circumstances (C2,V2); i.e., $C_1V_1 = C_2V_2$). One such method involves the patient breathing a known volume and concentration of an inert gas in a closed-circuit. Once equilibrium has occurred, end-expiratory gas concentration is used to measure lung volume. Another method is to have the patient breathe in pure oxygen and wash-out the resident nitrogen (N_2) in the lung, which essentially involves applying the same principle of conservation of mass. However, inert gas dilution only measures lung volume that is communicating with the mouth/atmosphere and therefore severe airway narrowing and/or airway closure can substantially underestimate lung volumes calculated by this technique.

Single Breath Nitrogen Washout (SBNW)- Closing Volume, Closing Capacity

The measurement of airway closure based on the single breath nitrogen washout (SBNW) test is founded on the principle that there is a minimum critical pressure below which terminal bronchioles close trapping gas within the lung [108]. The absolute lung volume at which an airway reaches this critical closing pressure is not homogenous as a result of pleural pressure gradients due to gravity and interdependence between lung regions. However, the volume range over which airway close is known as the closing capacity (CC), usually expressed as a percentage of TLC. When combined with measurement of RV, the absolute lung volume at which airways begin to close, termed closing volume (CV), can be calculated i.e. CV = CC – RV, and is usually expressed as a percentage of VC.

The SBNW test is performed by inhaling 100% O_2 from RV to TLC before expiring at a constant rate from TLC back to RV (Figure 5). At RV, the distribution of lung volume is dependent upon a gravity-related gradient so that prior to the inhalation of 100% O_2 lung volume is greater in upper lung zone > mid-lung zone > lower lung regions [177]. This leads to an apical-basal concentration gradient for N_2, with greater N_2 concentration in the upper lung than lower lung zones. Furthermore, inspiration is preferentially distributed to the upper lung zones up to ~20% of VC [177] so that preferential distribution of the anatomic dead space gas, which does not contain O_2, further contributes to a greater nitrogen concentration in upper zone airways. Then, as expiration from TLC begins, the first gas out is anatomic dead space still filled with 100% O2 and thus no N_2. Gas then comes from all regions of the lung, with varying mixtures of O2 and N_2, as expiration proceeds. As expiration approaches RV, the gravity-dependent differences in pleural pressure mean that airways in lower lung regions will begin to close (earlier) at higher absolute lung volumes. This onset of airway closure produces a rise in the expiratory N_2 concentration as expiration now more heavily reflects airways in the upper lung zones with a higher N_2 concentration.

Measurement of N_2 concentration throughout the entire expiratory trace provides the traditional four phase expiratory trace as described by Fowler [73]. Phase I represents dead-space and contains only 100% O_2, Phase II is the transition from dead-space to alveolar gas and Phase III is alveolar dead space with the slope of this "plateau" reflecting the extent of ventilation heterogeneity. At the end of Phase III there is the aforementioned sudden increase in N_2

concentration which is the Phase IV slope. The inflection point between Phases III and IV is CV and the volume over which Phase IV slope occurs is the closing capacity.

Peripheral Airway Resistance – Catheter Method

Airway closure has been inferred from direct measurement of changes in peripheral airway resistance (Rp) using the wedged bronchoscope technique. Kaminsky and colleagues modeled the change in Rp as a function in time following cessation of airflow through the wedged bronchoscope [121]. The plateau of pressure remaining within the wedged segment is assumed to be due to airway closure. In modeling of the response of the lung periphery to the direct instillation of cool, dry air to mimic hyperpnea, asthmatic participants were found to have higher Rp and plateau pressure both at baseline and in response to cool, dry air than healthy, control participants. These findings implicate increased narrowing and closure of collateral channels both at baseline and in response to cool, dry air in the wedged segment in asthma.

Forced Oscillation Technique – Changes in Elastance

The forced oscillation technique applies pressure oscillations to the airway opening in order to measure respiratory system impedance. This can be further partitioned into its components of respiratory system resistance and reactance, with the latter reflecting the contribution of compliance (reciprocal of elastance) and inertance. Respiratory system compliance comprises both lung tissue compliance, which equals the sum total compliance across parallel alveolar units, and the extent of gas compressibility within the lung. Therefore, one would expect that a

larger lung, due to more alveolar units in parallel and greater extent of gas compression would result in increased compliance (reduced elastance/less negative reactance). Indeed, population predicted equations for reactance include height as a surrogate of lung volume [36]. Similarly, one would expect that airway closure, which removes the tissue compliance and gas compression contributions of alveolar units, would lead to a reduction in compliance (increase in elastance/more negative reactance). This is supported by computational modelling in which changes in oscillatory compliance were only minimally affected by airway narrowing but were extremely sensitive to heterogeneous peripheral airway closure [159, 253]. (Figure 6). In addition, data from animal models have shown that measures of compliance/elastance correspond to the extent of airway closure measured by lung imaging [62, 158]. In humans, expiration from TLC to RV reveals a critical volume below which the magnitude of reactance dramatically worsens (becomes more negative) and it is thought that this volume corresponds to closing capacity [186] (Figure 7). Consistent with this is the finding that reactance measured during tidal breathing is more negative in obese subjects in whom FRC is below CC [166], suggesting that reactance is sensitive to airway closure and/or expiratory flow limitation occurring during tidal breathing. In people without respiratory disease, there is an expected inverse relationship between reactance and FRC measured by plethysmography [178]. However, in patients with COPD, in which airway closure is a characteristic feature, reactance was not correlated with FRC but was instead correlated with expiratory reserve volume and alveolar volume. These findings suggest that reactance is, in part, determined by the extent of lung volume communicating with the airway opening, further supporting the use of reactance as a measure of airway closure.

Imaging – CT, SPECT, PET, ^{3}He-MRI

There are several different imaging modalities for acquiring 3D volumes of the lung. These include Computed Tomography (CT), Single Photon Emission Computed Tomography (SPECT), Positron Emission Tomography (PET) and ^{3}He-Magnetic Resonance Imaging (MRI) [137] . CT imaging is based upon differences in density between the airway-filled lung and surrounding anatomic structures. After defining the outline of the lung based on a standard Hounsfield unit, CT lung volume is measured as the total number of voxels × voxel volume. As such, CT lung volume measures all volume within the lung at that specific lung volume, regardless of whether it is communicating with the airway opening or trapped behind closed airways. Indeed, there is no systematic difference between lung volume calculated from CT acquired at FRC and FRC measured by body plethysmography [69]. In contrast, SPECT and ^{3}He-MRI imaging involve inhalation of a specific contrast agent which enters ventilated airways and thus reflects the volume of lung in communication with the airway. Both have yielded impressive images of the distribution of ventilation (Figures 8, 9). PET on the other hand involves tracing nitrogen washout that has been injected intravenously as ^{13}N, and thus reflects both blood flow and ventilation. Following image segmentation to provide an outline of the lung, either guided by CT or by semi-automated software, ventilated lung volume and non-ventilated lung volume (also known as ventilation defects) can be calculated. It is therefore not surprising that there is no systematic bias between ventilated lung volume measured at FRC by SPECT and FRC measured by nitrogen washout [69]. However, it should be noted that not all ventilation imaging modalities are equal. SPECT imaging involves inhalation of Technegas, a 100nm radiolabeled carbon particle, which follows gas distribution during inhalation but “sticks”

to the lung where it remains for at least 20 min [3]. As such, SPECT reflects the ventilation distribution during inhalation and is minimally affected by the supine posture required for imaging. In contrast, ^{3}He-MRI ventilation imaging requires inhalation of a contrast agent at the time of image acquisition. Therefore, ^{3}He-MRI imaging reflects the distribution of ventilation while in a specific, horizontal position; i.e. supine, decubitus or prone posture. Interestingly, a recent study reported that the volume of lung affected by constriction following methacholine challenge was not different when methacholine was inhaled in the prone or supine posture, or whether imaging was performed supine or prone suggesting that posture during inhalation of spasmogen doesn't determine ventilation pattern [78]. In contrast, the posture during imaging determined the pattern of ventilation pattern.

Structural-Functional Correlation of Airway Closure

The significance of imaging findings in asthma (structural) may be appreciated by correlating such findings with those of functional measurements like FOT and MBNW. Such functional imaging studies have validated the importance of airway closure in asthma. For example, Farrow and colleagues used SPECT and MBNW to assess the response AHR to methacholine, and found that methacholine resulted in airway closure as seen by loss of ventilation on SPECT which was determined by the baseline level of ventilation heterogeneity in peripheral conducting airways (pre-methacholine Scond) (Figure 9) [69]. The authors also found that the increase in the proportion of poorly ventilated lung regions was associated with the change in ventilation heterogeneity in the peripheral acinar airways (increase in Sacin) [70]. These combined findings directly implicate airway closure and heterogeneous airway narrowing by both imaging and

MBNW. Likewise, Tgavalekos and colleagues have demonstrated that closure or near-closure of small airways must occur to explain the simultaneous findings of ventilation defects by PET images and the changes in lung resistance and elastance between 0.15 and 8 Hz [250].

Mechanisms of Airway Closure

Clearly, airway narrowing may lead to airway closure if the narrowing results in complete obliteration of the airway lumen. However, multiple factors are involved in determining whether an airway will close. First one must consider the forces causing airway narrowing. These include the bronchoconstriction caused by ASM constriction, and the narrowing of the lumen by airway wall thickening, which itself may be related to airway inflammation, remodeling or edema. In addition, any thickening of the airway wall will enhance the ASM constrictive response by geometric considerations [283]. Second, the surface tension of the airway lining fluid plays an important role in maintaining airway patency [296]. As the radius of curvature of the airway lumen decreases, the surface tension of the lining fluid increases according to the Law of LaPlace. However, surfactant within the lining fluid helps to stabilize the airway by lowering the surface tension as the radius decreases. The plasma proteins associated with inflammation in asthma have been shown to reduce the ability of surfactant to lower surface tension, making it more likely that the airway lumen may collapse shut. In addition, bridging fluid may join the walls and result in airway closure [288]. Thus, airway closure may be caused by not only simple bronchoconstriction but also by fluid bridging and surfactant dysfunction.

Consequences of Airway Closure

Airway closure or near closure will have a number of clinical consequences. As discussed, airway closure will lead to gas trapping and hyperinflation, which will markedly increase the work of breathing and worsen shortness of breath. In addition, the normal hyperpnea associated with exercise will result in dynamic hyperinflation, worsening dyspnea on exertion and reducing exercise tolerance. Airway closure is associated with more severe asthma [239, 240], risk of exacerbations [240] and poor control [130, 210]. Additionally, one would expect airway closure to have important implications for asthma treatment, since closed airways would not allow access for inhaled aerosol therapy [270, 276].

Ventilation Heterogeneity

Ventilation heterogeneity refers to the unevenness of airflow and ventilation that occurs throughout the lung. Since airway narrowing and airflow limitation occur in an uneven fashion in asthma, ventilation heterogeneity is an inevitable finding. Ventilation heterogeneity has important consequences in asthma.

Detection of Ventilation Heterogeneity

Spirometry- Flow-Volume Loop Shape Parameters

Uneven ventilation is thought to contribute to the shape of the flow volume curve. In intubated infants, the concavity of the flow-volume loop related to the degree of ventilation heterogeneity

[34]. New spirometric indexes related to the shape of the flow-volume curve have been ascribed, in part, to ventilation heterogeneity in COPD [20].

Single Breath Nitrogen Washout (SBNW) – Slope Phase 3

The SBNW test has been described above in relation to detecting airway closure (Figure 5). Ventilation heterogeneity is thought to be reflected in the slope of Phase III of the washout curve. If ventilation were entirely even, then all lung areas would empty simultaneously and evenly, and slope of Phase III would be flat. However, as time constants vary throughout the lung, some regions will empty faster than others, resulting in a positive slope of Phase III. An increased slope of Phase III has been described in poorly controlled asthma [29]. Since there is wide variability in the measurement of the slope of Phase III, it is felt to not have much clinical utility, but may still be valuable as a research tool.

Multiple Breath Nitrogen Washout (MBNW) – LCI, Scond, Sacin

Unlike SBNW, the MBNW is performed during quiet breathing of oxygen (Figure 10). Recent international guidelines have been published highlighting the importance of standardized technique. In this method, the slope of Phase III of each breath is successively measured over time, and slopes corrected for N_2 concentration are plotted against the cumulative volume of gas exhaled adjusted for FRC (called "lung turnovers"). The slope of this plot between lung turnovers 1.5 and 6 is felt to represent the heterogeneity of ventilation at the level of the peripheral conducting airways, and is termed Scond. The slope Phase III of the first breath

(minus the contribution of Scond at this point, numerically equal to Scond x lung turnover at first breath) is felt to represent the heterogeneity of the ventilation at the level of the junction between convective and diffusive gas flow occurring at the entrance to the acinar region, and is termed Sacin (Figure 11, 12). In asthma, Scond and Sacin are associated with other measures of ventilation heterogeneity obtained by imaging or FOT, and with clinical outcomes such as asthma severity and asthma control [65, 68, 70, 94, 245, 268]. Another global measure that is calculated is the lung clearance index, LCI, which represents the number of lung turnovers necessary to clear nitrogen down to 1/40th of it starting value. LCI is elevated in children with asthma despite good symptom control [218], but LCI is not able to discriminate proximal vs. peripheral ventilation heterogeneity like Scond and Sacin [272]. Interestingly, when SBNW and MBNW were compared in asthmatic subjects, MBNW indexes of uneven ventilation (LCI, Scond, Sacin) detected more abnormalities, but slope Phase 3 from SBNW was better associated with overall asthma severity [141].

Comparison of Alveolar Volume by Single Breath to TLC by Inert Gas Washout or Body Plethysmography (VA/TLC)

A measure of ventilation heterogeneity that is commonly obtained during routine pulmonary function testing is the VA/TLC ratio when both single breath DLCO and lung volumes are measured. Since the VA is measured by gas dilution during a 10 second breath hold at TLC, VA should nearly equal TLC once any equipment and anatomic dead space is subtracted. In a healthy lung, VA is approximately at least 85% of TLC. When VA/TLC is < 85%, VA is underestimating TLC likely due to insufficient time for gas distribution during the brief 10

second breath hold, which will be common among patients with uneven ventilation. We have found that VA/TLC is correlated with AHR among general patients presenting to the clinical PFT lab [122].

Force Oscillation Technique- Frequency Dependence of Resistance and Reactance

The FOT also allows insight into ventilation heterogeneity. Based on computational modeling, low frequency (< 5Hz) impedance reflects more peripheral lung mechanics, with signals in the <1 Hz range reflecting mechanics of the small airways and tissue [159]. Frequency dependence of resistance is seen at <1 Hz and is thought to be due to tissue viscoelasticity as well as ventilation heterogeneity [14]. The same is true of reactance at low frequency, particularly < 1 Hz.

Imaging – CT, SPECT, PET, ^{3}He-MRI

Ventilation heterogeneity is perhaps best appreciated by various imaging modalities. Uneveness of ventilation has been shown by CT scan, SPECT, PET and ^{3}He-MRI (Figures 8, 9). Each of these modalities has its own important strengths and limitations, but these methods provide important insight into ventilation heterogeneity in asthma and other lung diseases [134]. In conventional CT scan, Kaminsky and colleagues have shown that airspace heterogeneity, as a surrogate for ventilation heterogeneity, was increased following methacholine challenge [125]. More recently, Dame-Carrol and colleagues demonstrated increased heterogeneity of airway narrowing by CT in asthmatics, again implicating increased ventilation heterogeneity [59]. PET

imaging takes place as N^{13} is washed out of the lung following intravenous injection during apnea. These data provide information on both regional ventilation and perfusion and has yielded important insights through computational modeling about the size and location of airway narrowing and closure in asthma [249, 250]. SPECT is based on the topographic distribution of inhaled radiolabeled particles, such as the ultrafine carbon particle aerosol Technegas, or gases, such as xenon-133, and was one of the first techniques to demonstrate heterogenous ventilation in asthma [136]. ^{3}He-MRI relies on inhalation of ^{3}He, which being a gas can spread by convection and diffusion. In addition to demonstrating heterogeneous ventilation and drop out of ventilation thought to be due to airway closure [157], ^{3}He-MRI imaging allows an estimate of the dimensions of the alveolus as well as the degree of collateral ventilation using a measure known as the apparent diffusion coefficient (ADC) [277]. Recently, ventilation heterogeneity has been demonstrated by ^{3}He-MRI to be common to both healthy and asthmatic patients following methacholine-induced bronchoconstriction, but the asthmatics were unable to reduce heterogeneity following a DI [258].

Mechanisms of Ventilation Heterogeneity

Uneven distribution of airway narrowing will result in ventilation heterogeneity. Many factors may be involved, which include uneven ASM function along the airway tree [162, 163], variability in the degree of airway wall inflammation detected by exhaled NO in proximal vs. distal airways [216], and variability in the nature of underlying anatomic inflammation and structural remodeling of the airway wall [200]. Gravity itself results in uneven ventilation and blood flow throughout the lung, even in health, although heterogeneity of both ventilation and

perfusion persist even in microgravity, indicating additional mechanisms [215]. Furthermore, because the airways and lung parenchyma are interdependent, the distribution of ventilation is determined by the complex pattern of airflow in the lung. One phenomenon that has been described is the avalanche behavior of the lung in the presence of uneven distribution of airflow and/or mechanical properties that can lead to sudden and catastrophic airway narrowing and closure [152, 269]. How such airflow evolves cannot be predicted by analysis of the simple sum of all the airways in the lung, and instead is a manifestation of the emergence properties of the lung behaving like any complex system [290] (Figure 13). The combination of structural variability and functional variability determined by emergent properties was recently described by Donovan [64]. Finally, it is important to consider that ventilation heterogeneity is no doubt influenced by the uneven distribution of bronchoconstrictive agonists as they are inhaled into the lung [276].

Consequence of Ventilation Heterogeneity

Ventilation heterogeneity in asthma has significant clinical consequences. First, ventilation heterogeneity may lead to increased work of breathing by causing a functionally "stiff" lung [118]. In the case of uneven ventilation, some airways are more narrow than others, offering higher resistance to airflow, but at the same time more distended airways are stiffer, offering less compliance to accommodate gas flow. Because of uneven ventilation, the apparent stiffness of the lung increases with increasing respiratory frequency, a phenomenon known as frequency dependence of compliance. This translates into dyspnea and increased work of breathing at higher respiratory rates, such as would be seen with exercise. Second, ventilation heterogeneity

may predispose to abrupt development of ventilation defects from airway closure, which may lead to sudden worsening of asthma [290]. These defects may emerge as a consequence of the complex structure of the airway tree and the uneven distribution of airway narrowing. Ventilation heterogeneity, especially in the context of acute bronchospasm, also leads to imbalances in the ratio of ventilation (V) to perfusion (Q) [220]. In particular, the development of more low V/Q units in the lung leads to overall worsening of gas exchange, leading to hypoxemia and potentially hypercarbia. In response, the drive to breathe results in increased ventilation, which is best accomplished by increased respiratory rate because tidal volume recruitment becomes more and more limited by dynamic hyperinflation. This chain of events sets up a vicious cycle of increased drive to breathe, increased respiratory rate, decreased time for emptying, increased gas trapping and development of dynamic hyperinflation. Worsening dynamic hyperinflation leads to increased work of breathing, and the reliance on respiratory rate leads to rapid, shallow breathing and increased ratio of physiologic dead space to tidal volume ratio. This, in turn, leads to less ventilatory efficiency, and ultimately to CO_2 retention, which can further increase the drive to breathe. Thus, there ensues a worsening cycle of gas exchange that can ultimately lead to respiratory failure.

Third, ventilation heterogeneity may contribute to AHR, which is discussed in more detail below in the section on AHR. Fourth, ventilation heterogeneity is strongly linked to many aspects of asthma severity and control [2, 8, 67, 157, 245], perhaps through its influence on AHR, as well as its fundamental role in leading to the avalanche-like development of airway closure, as described earlier. In addition, the response of lung function to deep inhalation is variable in asthma, and computational modeling suggests that the size and frequency of deep breaths plays

an important role in determining the effect in the context of underlying heterogeneous airway narrowing and ventilation [83]. Finally, similar to the issue with airway closure, ventilation heterogeneity would be expected to have important effects on aerosol deposition, limiting the efficacy of this form of treatment in asthma [276].

Airway Hyperresponsiveness

Airway hyperresponsiveness (AHR) is a characteristic feature of asthma, and yet the term encompasses multiple definitions and methods by which it is measured. Ultimately different definitions/measurements are likely influenced by different pathophysiologies and as such, it is important to understand the differences before discussing the potential underlying mechanisms. Woolcock et al [293] administered increasing doses of an ASM agonist to asthmatic and healthy participants and reported two distinct differences in the nature of the dose response curve (Figure 14). Firstly, asthmatic participants had an increased sensitivity to response, leading to the leftward shift in the dose-response curve. Secondly, the dose-response curve in asthmatic participants was characterized by excessive bronchoconstriction, whereby there was no measurable response plateau. This excessive airway narrowing was equivalent to that seen in vitro [241]. On the other hand, a maximal response plateau was reported in healthy participants, in which large doses of agonist minimally reduced lung function. Therefore, the term AHR is used to describe the combination of the left-ward shift in the dose-response curve and the increase in the maximal response, whereas hypersensitivity is used to specifically denote the leftward shift in the dose-response curve [242]. Hypersensitivity can be measured as a lower dose that causes a pre-specified response, usually a 20% fall in FEV1, and can be measured as

the provocative dose (PDFEV1) [51]. Similarly, the fall in FEV1 can be plotted on a linear dose axis in order to calculate the dose-response slope, providing a measure of AHR in all subjects, and not just those whose response meets the pre-specified threshold [222]. In contrast, the maximal response plateau, or the lack thereof, is rarely measured in patients with asthma, either in research or clinical settings, and it is often not considered in animal models of allergic airways disease. Nonetheless, it is important to remember that AHR encompasses both hypersensitivity and loss of the response plateau when considering potential underlying mechanisms of AHR in human asthma.

Types of AHR - Direct vs. Indirect challenge

Airway hyperresponsiveness can be measured by stimuli which directly act upon effector cells leading to airflow obstruction (direct stimuli), or by stimuli which act upon intermediary cells which then act upon effector cells to cause airflow obstruction (indirect stimuli) [263] (Figure 15). The most common direct stimuli are histamine and methacholine, a synthetic mimetic of acetylcholine. Histamine acts through H1 receptors on airway smooth muscle to cause contraction and H2 receptors to cause the release of mucous [229]. H4 receptors have recently been discovered on hematopoietic cells [183], although it is likely that any contribution to asthma pathophysiology is due to effects on inflammation rather than on acute airway hyperresponsiveness. Methacholine stimulates muscarinic 3 receptors on airway smooth muscle to cause contraction and antagonizes relaxation through muscarinic 2 receptors [85]. Both histamine and methacholine likely contribute to airway narrowing through vagal reflex and local neurally-mediated mechanisms, although the contribution to AHR is unclear [231, 274]. At

present the role of muscarinic 1 receptors in response to methacholine in humans is unknown, although they appear to contribute to airway responses in rabbits [256] and parenchymal responses in dogs [237]. Furthermore, the release of mucous by direct stimuli is at least in part mediated by the airway epithelium [145]. Interestingly, maximal airway narrowing following methacholine can be further increased by histamine inhalation, suggesting differences in mechanisms or synergistic effects of the two direct stimuli [243]. However, AHR to direct stimuli is not specific to asthma, and has been reported in patients with chronic obstructive pulmonary disease [260], cystic fibrosis ([262]) and cardiac disease [226].

Indirect stimuli cause the release of mediators that mimic the inflammatory airway environment characteristic of asthma. Indirect stimuli are therefore thought to better reflect pathophysiology specific to asthma [90]. Indirect challenges can be split into those which employ a single strong stimulus, such as exercise and eucapnic voluntary hypernoea (EVH), and those which employ graded stimuli, such as hypertonic saline, mannitol, adenosine-5′-monophosphate (AMP) and allergen [90]. The development of indirect tests of AHR was based upon the recognition that exercise could cause asthma exacerbations and that the drug disodium cromoglycate could reduce exercise induced bronchoconstriction despite no direct action on airway smooth muscle [5]. The high ventilation during exercise and EVH increases the volume of air that is humidified during passage to the lower airways, leading to the loss of respiratory water and heat. As such, the major determinant of the response to exercise and EVH is the intensity of ventilation achieved. The loss of water triggers mucosal dehydration and an increase in osmolarity of the liquid surface lining the airways [254]. In response to the increased osmolarity, prostaglandin D_2, cysteinyl leukotrienes and potentially histamine are released into the airways by resident

structural and inflammatory cells, particularly mast cells and eosinophils [33, 91, 119]. These mediators then act on airway smooth muscle to cause contraction and airway narrowing. Exercise does not appear to recruit inflammatory cells into the airways suggesting that indirect tests reflect the resident inflammation environment [91]. Activation of sensory nerves may also contribute to exercise-induced bronchoconstriction although there is limited evidence in humans [111].

The other indirect stimuli cause bronchoconstriction at various levels of the aforementioned pathway. Hypertonic saline and mannitol challenges initiate bronchoconstriction by directly increasing the osmolarity of airway surface liquid leading to activation of mast cells and subsequent release of mediators [32, 167]. Similarly, AMP also likely activates mast cells to initiate the inflammatory cascade leading to airway smooth muscle contraction. Allergen challenge replicates both T-helper type-1 (innate) and type-2 (adaptive) inflammation through interaction with the airway epithelium and subsequent recruitment of inflammatory cells [48] as well as activation of mast cells, basophils and eosinophils through antigen-IgE complexes.

The dependence of indirect AHR on the release of inflammatory mediators suggests that they may better reflect underlying inflammation than direct challenges. Accordingly, levels of exhaled nitric oxide and sputum eosinophils are more closely correlated with the severity of AHR measured by indirect tests than by methacholine [18, 212]. Similarly, the more rapid response to inhaled corticosteroid treatment of indirect AHR compared to direct AHR suggests that indirect tests may better reflect underlying inflammation [66]. While AHR to mannitol has been reported in patients with mild-moderate COPD, the strong association with biomarkers of

eosinophilic airway inflammation further supports the association between indirect challenge AHR and underlying active inflammation [61]. There is a moderate correlation between the severity of AHR measured by mannitol and histamine, although there is a high discordance between AHR defined by direct and indirect stimuli [133, 154]. However, patients with AHR to both indirect and direct stimuli have the greatest risk of exacerbation following down-titration of inhaled corticosteroid treatment [154], again suggesting that they reflect, at least in part, different and additive pathophysiology.

Airway Responsiveness as a Continuous Variable

AHR is not a binary classification but rather the extreme end of a non-normal distribution [203]. AHR is present in 7-20% of the general population [189, 202]. The severity of airway responsiveness is at least partially fluid. For example, in patients with asthma, an increase in the severity of AHR can occur during seasonal allergen exposure or after acute allergen challenge [27, 52]. Similarly, airway responsiveness can be increased in normal healthy subjects through the avoidance of deep inspiration, reduction in functional residual capacity, supine posture, and in the pathological setting of obesity. Each of these interventions provide important insight into the pathophysiology of AHR and therefore will be considered as an introduction to understanding the mechanisms of AHR in asthma.

The Effect of Deep Inspiration Avoidance on Airway Responsiveness

Normal tidal breathing predominantly involves breaths of relatively small volume compared to total lung capacity (TLC). However, these are periodically punctuated by breaths of increased volume, from slightly greater than normal up to full inspiratory maneuvers, referred to as deep inspirations (DIs) [28]. DIs of at least three times tidal volume occur every six minutes [17] and are due to a vagally mediated mechanoreflex which is both initiated and regulated by afferent input from peripheral chemoreceptors [11]. These DIs have two important effects in health: they protect against subsequent bronchoconstriction (bronchoprotection) and reverse existing bronchoconstriction (bronchodilatation) [46]. The extent of bronchodilatation is reduced in asthma due to both an inability to dilate airways and increased rate of re-narrowing [224]. In contrast, DI bronchoprotection is completely lost in asthma [234] and in subjects with AHR who do not have asthma [227], suggesting that the loss of DI bronchoprotection is one of the primary causes of AHR.

The increase in airway responsiveness in normal healthy subjects following DI avoidance was initially speculated to be due to the loss of large length excursions necessary for normal airway smooth muscle function [234]. This was based on in vitro experiments showing that the airway smooth muscle is highly plastic and that force-generating capacity can be acutely reduced in response to acute alterations in resting length [279]. This is thought to be due to structural re-arrangement of the contractile elements of airway smooth muscle [88] or alterations in cross-bridge dynamics [88]. However, the profound effect of length perturbations on ASM strips in vitro was not replicated when experiments were scaled to whole airway segments undergoing cyclical stretching matching normal breathing [6, 187]. This is consistent with evidence that DI avoidance in healthy people does not increase airway narrowing measured as respiratory system

resistance (118), specific airway conductance or partial expiratory flow (31). In contrast, Lutchen et al [160] reported that avoiding DI increases the response to methacholine as measured by elastance at 8Hz, as well as resistance and elastance at very low frequencies down to 0.1Hz, reflecting peripheral airways and lung tissue. Computational modelling suggested this was due to heterogeneous constriction involving airway closure or near closure. This was later confirmed by Chapman et al [45] who reported that DI avoidance led to increased airway closure, but not airway narrowing, during subsequent methacholine challenge.

The mechanisms underlying DI bronchoprotection are currently unclear, but avoiding DI itself does not alter respiratory system resistance, respiratory system reactance, specific airway conductance, residual volume or partial expiratory flow [45, 57]. Instead, it is likely that avoiding DI somehow 'primes' the airways for an increased response once the airway smooth muscle is stimulated. Continuous tidal volume ventilation without Dis leads to abnormal surfactant structure and increased surface tension in rabbits suggesting that DI avoidance may disrupt normal surfactant homeostasis and predispose to airway closure [98, 252]. Indeed, distortion of alveolar cells by DI causes the release of pulmonary surfactant in rats [184] and if the release of surfactant in healthy subjects is similarly dependent upon distortion, then DI avoidance could result in an inadequate supply of surfactant. A role for surfactant in DI bronchoprotection is consistent with the finding that bronchoprotection is dramatically reduced when the DI is expired to residual volume (instead of to FRC) [45], since expiration to low lung volume causes the loss and/or deactivation of surfactant molecules [267]. Together, these findings lead to the speculation that DI avoidance alters peripheral airway properties, potentially

due to surfactant dysfunction, that then predisposes to increased airway closure and increased AHR.

The Effect of Lung Volume on Airway Responsiveness

The effect of lung volume on airway responsiveness has provided considerable understanding to the pathophysiology of AHR in asthma as well as to the link between asthma and obesity. Reducing end expiratory lung volume leads to increased airway responsiveness in healthy subjects whether done by voluntary breathing at low lung volumes, supine posture or increasing the external load on the chest wall [44, 63, 175]. This effect is seen as a substantial increase in the maximal response plateau, without any alteration in sensitivity [63]. While the mechanisms are not fully understood, three hypotheses predominate in the literature. Firstly, low lung volume breathing may allow airway smooth muscle to adapt to a shorter length thereby generating increased force and greater airway narrowing [214]. Indeed, reducing FRC by ~25% in sheep with rigid chest strapping increased the in vitro rate of stress generation in airway smooth muscle [171]. Secondly, a reduction in FRC would reduce the outward tethering forces exerted on the airways by the lung parenchyma [173]. The interdependence between the airways and parenchyma maintains airway caliber so that a reduction in this force would be expected to contribute to airway collapse, especially during airway narrowing. Both voluntary low lung volume breathing [109] and chest wall strapping increase ventilation heterogeneity [44] which computational modelling predicts would predispose to localized and exaggerated airway closure upon ASM stimulation (134). Indeed, chest wall strapping leads to exaggerated airway closure during bronchial challenge [206]. Interestingly, the reduction in lung volume and increase in

ventilation heterogeneity independently contribute to the increase in airway responsiveness with chest wall strapping [44] suggesting that the effects of ventilation heterogeneity on AHR in asthma may be independent of alterations in ASM function and airway-parenchymal interdependence.

In obesity, increased adiposity exerts a force on the lungs that shifts the balance between the inflationary and deflationary pressures [223], leading to the characteristic reduction in functional residual capacity (FRC) [117]. As discussed above, lung volume has long been recognized to regulate airway responsiveness and therefore this reduction in FRC has been widely speculated to underlie AHR in obese subjects. In obese asthmatics with little to no evidence of allergic inflammation (T_H2-low), weight loss substantially improves AHR due to a reduction in peripheral airway closure [1, 47]. A recent computational model suggested that natural variation in properties of the airway would predispose certain individuals to AHR when airway-parenchymal interdependence is reduced, therefore explaining why some, but not all, obese people have AHR [13]. Additionally, the reduction in FRC in obesity can be so extreme that tidal breathing occurs at or below closing volume [166] suggesting that obesity may increase ventilation heterogeneity [56]. However, in non-asthmatic subjects, airway closure appears confined to the basal lung zones [100] and therefore obesity may not increase ventilation heterogeneity in people without respiratory disease. Interestingly, BMI, but not waist circumference, correlates with the extent of airway closure during bronchoconstriction [211], suggesting that AHR due to obesity may not be caused by mechanical mechanisms. The authors speculated that the increased airway closure in obesity may be due to factors associated with subcutaneous adipose tissue, such as surfactant dysfunction [112]. Thus, while breathing at low

lung volumes appears to be the primary reason obesity is linked to AHR, there are other aspects of obesity that likely play important roles as well.

Mechanisms of AHR

Multiple mechanisms are implicated in the phenomenon of AHR (Figure 16).

Airway Smooth Muscle

Bronchoconstriction is due, at least in part, to contraction of the airway smooth muscle (ASM) leading to airway narrowing. Therefore, it is not surprising that dysfunction of the ASM has long been touted as the principle cause of AHR in asthma. Dysfunctional ASM could be due to intrinsic abnormalities of the ASM itself or to altered properties induced by the inflammatory environment in which it resides. It is currently not clear whether the ASM is intrinsically abnormal in asthma and, if it is, what factors may contribute to a hyper-contractile state. Birth cohort studies suggest that lung function and increased airway responsiveness are evident as early as 1 month of age [257, 298]. These abnormalities are unrelated to markers of airway inflammation [298] and may even occur before allergen sensitization [103] consistent with the hypothesis that intrinsic dysfunction of ASM may contribute to AHR in asthma. Similarly, there are distinct gene expression profiles between ASM from asthmatics and non-asthmatics, with the expression of four genes correlating with the severity of AHR [297]. The contribution of intrinsic abnormalities in ASM to AHR is further supported by the correlation between increased expression of the contractile proteins α-smooth muscle actin and desmin in ASM, and the

severity of AHR in patients with asthma [236]. Although studies have reported increased in vitro ASM forced generation in asthma [10, 22], this is not a consistent finding [49, 82]. Alternatively, an increase in ASM shortening velocity would lead to exaggerated airway narrowing even in the presence of normal force generation because a muscle that shortens (contracts) quickly would produce greater airway narrowing during expiration when the lung parenchyma is not opposing ASM shortening [238]. This is consistent with increased re-narrowing following full lung inflation in asthma [224]. Furthermore, in vitro experimentation has shown a link between shortening velocity and total amount of ASM shortening during oscillations mimicking tidal breathing [39]. Computational modelling suggested that this could either be due to increased rate of actin-myosin cross-bridge cycling or the rate of myosin light chain phosphorylation. The rate of actin-myosin cross-bridge cycling is dependent upon the activity of myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP), with reports of increased expression of MLCK in asthma [161]. However, more recent data has questioned whether shortening velocity is in fact increased in asthma [49]. Similarly, normal subcellular structure of the ASM in asthma [247] further questions the role of intrinsic ASM dysfunction in AHR.

The ASM need not to be intrinsically abnormal to contribute to AHR in asthma. We have already discussed the plasticity of the ASM in response to changes in length and there is substantial evidence to suggest that it is also highly adaptable to its environment within the airway wall. In asthma, this environment consists of numerous resident inflammatory cells and a milieu of pro-inflammatory cytokines. The number of mast cells within the ASM correlates with the severity of AHR in asthma [232], which is likely mediated by the effect of mast cell mediators on ASM activation [93]. Proteases, such as matrix metalloproteinase-1 (MMP-1), are also increased

within ASM bundles of asthmatics and regulate in vitro ASM contractility [221]. Interleukin-4 (IL-4), IL-13 and tumor necrosis factor-α (TNFα) have all been shown to increase ASM responsiveness in vitro [233]. These findings led Bosse et al to suggest that AHR in asthma is due to "a good muscle in a bad environment" [23]. This was based on a long line of evidence in which they showed that ASM adapted to an increase in basal tone to produce a synergistic increase in airway shortening in vitro [24] and increased airway responsiveness in both an animal model [153] and in healthy humans [77]. In contrast, considerable evidence suggests that an inflammatory environment may initiate a transition from a contractile ASM phenotype to a "synthetic" phenotype characterized by reduced contractile-associated proteins but increased proliferation and chemokine secretion [295]. However, it is unclear in what way and to what extent a synthetic ASM phenotype alters AHR in asthma.

Airway Remodeling

Airway remodeling refers to the structural alterations of the airway wall characteristic of asthma due to a combination of chronic airway inflammation [285], early life exposures [191] and intrinsic factors. These changes include subepitheial fibrosis [286], ASM hypertrophy/hyperplasia, angiogenesis and changes in extracellular matrix composition [114]. Each of these changes likely contribute to an increase in the thickness of the airway wall in asthma and subsequently contribute to AHR. Airway resistance is inversely related to airway radius (Equation 1), so that a reduction in the diameter of the airway lumen due to airway remodeling would amplify the extent of airway narrowing for any given degree of ASM shortening [148, 181]. Additionally, an increase in the thickness of the adventitial layer or

infiltration of inflammation into the adventitial interstitium has the potential to uncouple the interaction between the airway wall and the lung parenchyma. The airway and lung parenchyma are structurally linked allowing the transmission of the elastic recoil force of the lung parenchyma to the airway to maintain airway caliber. A loss in airway-parenchymal interdependence would reduce the load against which the ASM shortens, allowing the ASM to shorten more before being balanced by parenchymal tethering. Consistent with both mechanisms, the degree of airway wall thickening in asthmatics with airway obstruction has been shown to correlate with the severity of AHR [26]. Lastly, computational modelling suggests that an effect of airway remodeling on the way in which the basement membrane buckles could reduce the load opposing airway narrowing and thus contribute to AHR [147], although whether this contributes to in vivo AHR in asthma remains unknown.

Interestingly, there is a small body of evidence that airway remodeling in asthma may not increase AHR. On one hand, it has been suggested that airway remodeling, and the subsequent increase in airway stiffness, may actually oppose airway narrowing [172]. Milanese et al [176] reported reticular basement membrane was increased in patients with asthma and that the thickness was correlated with less severe AHR. Similarly, Niimi and colleagues reported that increased airway thickness measured by CT correlated with reduced airway hyperreactivity, suggesting that airway remodeling may limit excessive airway narrowing in patients with asthma. In yet another twist, recent evidence suggests that instead of being a cause of airway responsiveness, airway remodeling may actually be a consequence of airway responsiveness. In an elegant study, Grainge et al [86] showed that three episodes of bronchoconstriction due to either allergen or methacholine challenge was sufficient to induce similar levels of subepithelial

collagen deposition and mucous hyperplasia. These changes were not due to airway inflammation as there was no change in eosinophilic inflammation with methacholine challenge. Similarly, the effects were linked to bronchoconstriction because airway remodeling was not found in asthmatics who were administered albuterol to block airway narrowing. The underlying mechanisms are likely related to mechanotransduction in airway epithelial cells. Airway epithelial cells in vitro are sensitive to compressive forces equivalent to those experienced during bronchoconstriction and release numerous mediators linked to various features airway remodeling in asthma [198].

Airway-Parenchymal Interdependence

As discussed previously, the mechanical properties of the airways and lung parenchyma are structurally and functionally linked, known as airway-parenchymal interdependence. The elastic load provided by the lung parenchyma determines airway caliber [244] and provides a load against which the ASM must shorten [181]. Comparison of the distribution of ventilation between different postures has shown that the distribution of bronchoconstriction is gravitationally dependent, i.e. when changing from supine to prone posture, airway closure 'shifted' to the most dependent regions during bronchial challenge [95, 96]. This suggests that parenchymal distending forces are a strong determinant of bronchoconstriction because pleural pressure gradients due to gravity result in dependent lung regions that are less distended and thus exert less distending forces on the airways to resist airway narrowing and closure.

The importance of parenchymal independence on airway caliber and bronchoconstriction suggest that reductions in this elastic load may contribute to AHR in asthma. A reduction in the elastic load provided by the lung parenchyma may occur through three mechanisms. Firstly, damage to the alveolar attachments connecting the lung parenchyma and airways, as reported in fatal asthma [170], would result in a loss of transmission of the parenchymal forces opposing ASM shortening. Secondly, inflammation or accumulation of fluid within the adventitial interstitial space may impede the transmission of the elastic recoil of the lung to the airways i.e. effectively uncoupling the interdependence [164]. This is consistent with the loss of elastic recoil during an exacerbation of asthma which is normalized following resolution [292]. Thirdly, a reduction in the elastic properties of the lung parenchyma would directly reduce the outward force on the airways and may promote airway narrowing during ASM shortening. Although underappreciated, elastic recoil can be reduced in asthma as evidenced by mild centrilobular emphysema [80] and reduced lung density at total lung capacity [21]. While the loss of elastic recoil in asthma correlates with the extent of peripheral airway dysfunction [255], there is a lack of direct evidence that a reduction in loss of elastic recoil or loss of interdependence contributes to AHR in asthma.

Airway Epithelial Function

The airway epithelium is the first barrier for inhaled spasmogens and therefore any damage to the integrity of the epithelium would likely allow greater access to the ASM and exaggerate contraction. Indeed, mechanical removal of the airway epithelium in canine bronchial segments leads to increased sensitivity that is then comparable to sensitivity when the agonist is applied on

the adventitial surface [179]. Similarly, administration of cationic proteins to the airways of mice leads to damage to the airway epithelium [259] and increased airway sensitivity due to exaggerated ASM shortening [16]. Interestingly, cationic protein did not change the maximal response plateau [16]. In patients with asthma the severity of AHR correlates with increased epithelial damage measured quantitatively by biopsy [115] and reflected by the concentration of epithelial cells in sputum [91]. Furthermore, the airway epithelium is not merely a barrier but actively impacts its environment through secretion of multiple mediators. For example, release of epithelial-derived relaxing factor maintains relaxation of ASM [264]. Therefore it is not surprising that damage to the epithelium decreases its ability to reduce ASM activation [55]. Furthermore, epithelial cells may themselves contribute to airway narrowing since it has been shown that rupture of small airway epithelial cells in vitro induce intracellular [Ca2+] waves and subsequent contraction in neighboring ASM [299]. Similarly, epithelial cells exposed to mechanical compression equivalent to bronchoconstriction cause an endothelin-1 mediated increase in ASM contraction [149]. Lastly, damage to the airway epithelium is associated with an increase in inflammatory mediators, such as IL-33, which may contribute to AHR through exaggerated (eosinophilic) airway inflammation [89]. All the above mechanisms are likely especially relevant to the development of occupational asthma following inhalation of noxious molecules.

Neural Control

The airways are richly innervated by both the sympathetic and parasympathetic nervous system, so it is no surprise that neural input regulates airway tone and indeed is implicated in AHR.

There is strong evidence that cholinergic activity is increased in asthma and implicated in airway narrowing, as is seen clinically by the significant bronchodilating effect of anticholinergic aerosols such as ipratropium. Anticholinergics also markedly reduced AHR to a wide variety of stimuli [42]. In addition, nedocromil inhibits sensory nerve activation and prevents AHR to exercise and other irritants [50], implicating the non-adrenergic, non-cholinergic nervous system in the pathogenesis of AHR in asthma.

Heat and Water Loss

The main stimulus involved in AHR to exercise or hyperpnea is the heat and water lost during periods of high minute ventilation. The extensive airway tree serves to warm and humidify the inhaled air to body temperature and humidity by the time the air has reached the alveoli. At high levels of ventilation air that is not fully conditioned may penetrate deep into the airway tree and serve as the stimulus bronchospasm. The primary mechanism is thought to be due to drying of the airway lining fluid, leading to an increase in local ion concentration and hence osmolarity, which serves to stimulate epithelial cells, mast cells, eosinophils and sensory nerve cells that line the airway to release mediators [139]. The net effect of this release is bronchospasm, caused mainly by release of cysteinyl leukotrienes, PGD2 and neurokinins. The response may be modulated by the inspired air temperature, which if cool may cause bronchoconstriction more rapidly. Asthmatics with EIB appear to have a higher density of mast cells within their epithelial layer [146]. Damage to the normal biological function of the airway epithelium may compound EIB by interfering with normal re-humidification and allowing inhaled irritants easier access to underlying inflammatory cells.

Ventilation Heterogeneity

Recently it has been speculated that increased baseline ventilation heterogeneity may be a mechanism which could lead to AHR in asthma. At the anatomic level, it seems apparent that heterogeneity of inflammation throughout the lung, and of airway wall dimensions and ASM would contribute to AHR [200], and enhance AHR [81], as shown by computational modeling. However, ventilation heterogeneity seems to be important in determining AHR independent of inflammation [65]. Venegas et al (134) used positron emission tomography (PET) to show that during induced bronchoconstriction asthmatic subjects develop areas of severely under-ventilated lung. However, these ventilation defects were not totally devoid of ventilation, but rather included airways which contained normal levels of ventilation, suggesting that these ventilation defects were not due to closure of large airways alone. The authors developed a highly advanced lung model that takes account of the effects of the tethering forces of the lung parenchyma in which the airways are embedded, the intra- and extra-luminal pressures and the smooth muscle forces (134). The model predicts that uniform ASM contraction with the addition of small, random heterogeneities in airway caliber would lead to the abrupt development of airway closure when ASM contraction reaches a critical level of instability. The increase in airway narrowing and decrease in the volume of local alveoli results in the reduction in parenchymal tethering forces, initiating a short-range feedback mechanism whereby airway closure propagates up and down the airway tree. This development of large clusters of airway closure was consistent with the PET images and their model suggested that they would occur even with small levels of ASM stimulation. An increase in baseline ventilation heterogeneity

may provide sufficient airway instability to initiate this positive feedback mechanism. Increased baseline ventilation heterogeneity is not necessarily specific to one disease process, but rather could be induced by any process that leads to non-uniform airway caliber, such as airway remodeling, intraluminal exudate or reductions in elastic recoil. This theory therefore includes and combines the effects of the many abnormalities of asthma that may result in an uneven distribution of airway calibers throughout the airway, potentially explaining their role in both the increased sensitivity and excessive bronchoconstriction which are characteristic of AHR.

Parenchymal Lung Mechanics in Asthma

One of the most important means to detect changes in the functional properties of the lung parenchyma is by measurement of the pressure-volume (PV) curve. Typically the PV curve in stable asthma is normal or shifted upward, indicating normal or increased lung volumes but normal elastic recoil [53, 102]. During acute exacerbations of asthma, the PV curve may shift upward and to the left, indicating loss of elastic recoil [292]. The loss of recoil has been postulated to be due to stress-relaxation of the elastic elements in the lung parenchyma [102]. In most cases following the exacerbation, lung compliance appears to return to normal. There are also some asthmatics who have PV curves persistently shifted up and to the left, which suggests a chronic loss of recoil [79, 80]. The etiology of this loss of recoil is unknown, but there may be evidence of microscopic emphysema in such patients [80]. In some cases during acute exacerbation, the PV curve may shift down and to the right, with the apparent loss of lung volume and increased recoil likely due to airway closure [292], possibly from surfactant dysfunction. Surfactant is an important component of the alveolar structure, as it exists within

the alveolar lining fluid and serves to regulate surface tension. Surfactant function is disrupted by any type of protein presence, and since inflammation involves the elaboration of protein, it is not surprising that surfactant function may be compromised in asthma [99]. This would tend to reduce lung compliance and make it easier for narrowed airways to close, a mechanism that is thought to potentially contribute to the increased propensity for airway closure in asthma. Interestingly, ASM tone can increase parenchymal stiffness, presumably from transmission of retraction forces into the surrounding lung parenchyma [180]. This may the cause of so-called "reversible restrictive lung disease", in which an apparently stiff lung based on a rightward and downward shift of the PV curve can be brought back to the normal position following albuterol. One case report of this implicated increased ASM tone around the alveolar duct region, that resulted in an increase in overall lung stiffness [123]. Thus, there appear to be competing factors affecting the mechanical properties of the lung parenchyma, those that increase elastic recoil, such as surfactant dysfunction, and perhaps involving increased ASM tone in the airways or lung parenchyma, and those that decrease elastic recoil, such as from chronically elevated lung volumes and perhaps microscopic emphysema.

Relationship of Lung Mechanics to the Clinical Manifestations of Asthma

Many of the consequences of altered lung mechanics and how they relate to the clinical manifestations of asthma have been discussed. For example, airway narrowing leads not only to increased work of breathing from elevated airway resistance, but also increased work of breathing due to air trapping and hyperinflation, markedly affecting the symptom of dyspnea [155]. Heterogeneity of airway narrowing and ventilation leads to increased propensity for AHR

[44, 65, 67, 70, 94, 156]. Ventilation heterogeneity has also been linked to asthma control [68, 69]. Loss of lung recoil, whether anatomic or functional, may predispose to sudden, acute worsening or even near fatal asthma [79]. Recently, Antonelli and colleagues examined the mechanical correlates of dyspnea in asthma by carefully comparing symptoms to alterations in lung mechanics measured by the FOT in response to methacholine [7]. Low levels of bronchoconstriction caused dyspnea that were associated with dyspnea related to airway narrowing and loss of bronchodilation after DI, perhaps due to more central airway narrowing, whereas higher levels of bronchoconstriction were associated with dyspnea related to ventilation heterogeneity and loss of lung volume from airway closure, perhaps due to more peripheral airway narrowing [7].

Thus, it is clearly apparent that the symptoms and clinical manifestations of asthma extend well beyond the simple effects of airway narrowing and resulting increase in airway resistance (Figure 17). We are just now appreciating the importance of altered lung mechanics beyond airflow limitation in asthma, and may soon realize the clinical utility in measuring respiratory system impedance, ventilation heterogeneity, and other aspects of altered lung mechanics that may help us better care for our patients with asthma.

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Tables

Table 1 – Consequences of Airway Narrowing

- Wheezing
- Expiratory flow limitation
- Gas trapping and hyperinflation
- Ventilation heterogeneity
- Ventilation/Perfusion mismatching
- Airway Hyperresponsiveness

Figure Legends

Figure 1 – Illustration of major inflammatory mechanisms involved in asthma pathogenesis. The healthy airway is depicted at the top, with airways smooth muscle surrounding the airway epithelium sitting on the reticular basement membrane. Moving counterclockwise, eosinophilic asthma is seen by increased eosinophilic inflammation driven by allergen stimulation resulting in increased eosinophilic and mast cell activation. Next is seen non-allergic eosinophilic inflammation driven by pollutants and microbes also resulting in increased eosinophilic inflammation activation. In the lower right section is seen Type 1 and Type 17 neutrophilic inflammation, driven by pollutants, microbes and oxidative stress, resulting in increased neutrophil activation. Non-eosinophilic asthma may also be paucigranular (top right), with few inflammatory cells, or mixed granulocytic, with both eosinophilic and neutrophilic inflammation (bottom). From [195], with permission.

Figure 2 – Illustration of key factors determining airway narrowing. Airway caliber is regulated by surrounding airway smooth muscle force balanced against parenchymal tethering. Other factors that modulate airway caliber include the thickness of the airway wall due to inflammation, edema and remodeling, mucosal folding, and the elastic properties of the airway wall. From [97], with permission.

Figure 3 – Illustration of effects of deep inhalation (DI) on airway size based on the concept of relative hysteresis of airway and lung parenchyma. A) Airway and lung pressure vs. volume curves showing equal hysteresis of airway and lung, as seen by the area within the P-V curves.

In this circumstance, a deep inhalation results in no change in airway caliber, as seen by the symmetrical balance of Pressure for a given Volume on the left, and the equal diameters of the airways on the right. B) PV curves showing airway greater than parenchymal hysteresis, which results in a bronchodilator response to deep inhalation, as seen by the greater dilator force after inhalation. C) PV curves showing parenchymal greater than airway hysteresis, which results in a bronchoconstrictor response to deep inhalation, as seen by the greater constrictor force after inhalation. From [207], with permission.

Figure 4 – Diagram illustrating fluctuations in peak flow over time. This recording shows fluctuating peak flow measured twice daily over 150 days. The statistical pattern of fluctuation is similar at different time scales, as seen by the inset showing magnification of a shorter time period, illustrating the fractal properties of this time series. From [75], with permission.

Figure 5 - Single Breath Nitrogen Washout trace from a healthy 21 year old male without respiratory disease. Participant inhaled 100% O2 from residual volume (RV) to total lung capacity (TLC) and then expired from TLC to RV. The expiratory nitrogen trace is comprised of four distinct phases: phase I (dead space), phase II (bronchial ventilation), phase III (alveolar ventilation) and phase IV (sudden increase indicating onset of airway closure). Closing volume (CV, blue bar) is the expired volume between the onset phase IV and RV. Closing capacity (CC) is calculated as CV + RV. The slope of phase III is plotted between 25% and 75% of vital capacity (orange dashed line). Note the unconventional x-axis in which volume been plotted based on total lung capacity measured by body plethysmography to show CC and RV.

Figure 6 – Illustration of effects of homogeneous vs. heterogeneous airway constriction on Resistance and Elastance measured by the forced oscillation technique. Left, resistance is plotted against frequency and compared between the healthy state and different states of airway constriction. Under conditions of homogeneous constriction, there is relatively uniform elevation of R across the frequency range. Under conditions of heterogeneous constriction, there is marked frequency dependence, with R at lower frequencies being markedly elevated. Right, effects of different airway constriction patterns on elastance (E) relative to the healthy state. Homogeneous constriction causes most of the oscillatory input signal to be shunted into the central airways, resulting in a uniform increase of E with frequency. Heterogeneous airway constriction results in a marked rise in E with a jump in E at breathing frequency reflecting airway closure. From [156], with permission.

Figure 7- De-recruitment measured by forced oscillation technique in a 21 year old healthy male without respiratory disease. The participant performed a slow vital capacity during which respiratory system reactance (Xrs) was continuously measured and the points of de-recruitment (DR1 = diamond, DR2 = circle) calculated as previously described (Nilsen et al, JAP, 2019). Note that functional residual capacity (FRC) occurs above both de-recruitment points and that closing capacity (CC) measured from the Single Breath Nitrogen Washout test occurs at approximately the same volume as DR2.

Figure 8 – Imaging of heterogeneous ventilation by hyperpolarized helium (^{3}He)-MRI. Shown in this example are the ventilation images obtained from a healthy subject (A), compared to 3 different subjects with mild (B), moderate (C) and severe (D) asthma. Notice the increasing

number of ventilation defects (white arrows), reflecting functional airway closure, as asthma becomes more severe. From [225], with permission.

Figure 9 – Imaging of heterogeneous ventilation by ventilation SPECT/CT. Shown are the images from one subject before (left) and after (right) methacholine. Not only are there larger and new poorly ventilated or non-ventilated spaces (black), but the ventilation has become more heterogeneous within the ventilated airspaces, as seen by the color-coding of ventilation and the histogram distribution of ventilation below the images. From [70], with permission.

Figure 10 - Nitrogen trace from a Multiple Breath Nitrogen Washout test. The participant was instructed to take tidal volume breaths of approximately 1 liter until end-expiratory nitrogen concentration had fallen by 1/40th of the initial concentration (~2.5%). Lung Clearance Index (LCI) is calculated as the lung turnover (cumulative expired volume/functional residual capacity) at this point. Insets show the phase III slope *normalized* by the mean expiratory nitrogen concentration for the 1st and 25th breaths, respectively.

Figure 11 - Derivation of two parameters of ventilation heterogeneity from the multiple breath nitrogen washout test. The slope of the plot between lung turnovers 1.5 and 6 reflects ventilation heterogeneity occurring in airways where gas transport is dependent upon convection. This slope is termed Scond as it is felt to represent ventilation at the level of the peripheral conducting airways. The (normalized) phase III slope of the first breath (minus the contribution of Scond i.e. Scond x lung turnover at first breath) reflects ventilation heterogeneity at the interface between convection and diffusion gas transport, known as the diffusion front. This is felt to

represent the heterogeneity of the ventilation at the entrance to the acinar region, and is termed Sacin. Data are shown from a 34 year old male with normal lung function (△) and a 45 year old female with asthma (○).

Figure 12 – Illustration of anatomic areas of involvement in determining conduction-dependent and diffusive-conductive dependent ventilation. While not meant to precisely indicate anatomic location, Scond is seen to arise in more proximal conducting airways, and Sacin is seen to arise in more distal airways at the junction between conductive and acinar ventilation. From [271], with permission.

Figure 13 – Parallel and Serial Airway Interdependence Leading to Heterogeneous Ventilation. This illustration depicts a symmetrically bifurcating airway tree under uniform ASM constriction (red lightning bolts). On the left is illustrated the consequences of parallel interdependence of airways on ventilation. One airway has a slightly thicker airway wall than the other (red area). Accordingly, during ASM constriction, airflow is reduced more to this airway than its daughter branching airway (dashed arrow). The reduced airflow leads to reduced distal ventilation, and hence reduced expansion of the surrounding lung parenchyma. This leads to reduced tethering forces on the embedded airway, allowing less stretch of the ASM, and therefore enhancing its constriction. This pattern results in a vicious cycle (curving arrows) culminating in widespread loss of ventilation to the parenchyma fed by that airway ("catastrophic airway collapse"). This is seen as a ventilation defect seen on imaging (Vdef). Meanwhile, under conditions of conserved total minute ventilation, airflow is diverted away from the thickened airway into the daughter airway without airway wall thickening (thicker

arrow) increasing ventilation to the parenchyma served by that airway. This results in increased expansion of that parenchymal area with consequent increased tethering forces and increased tidal ASM stretch and dilation of the airway. This process also cycles until a dynamic equilibrium of airway size and parenchymal expansion is reached.

On the right is shown the consequences of serial interdependence of airways. Transmural pressure across the airway is greater for central than peripheral airways during tidal breathing, such that under conditions of uniform ASM constriction, central airways are stretched more during tidal breathing than peripheral airways, resulting in a net greater narrowing of peripheral airways than central airways. The end result may once again be ventilation defects seen on imaging. Adapted from [289], with permission.

Figure 14 – Illustration of airways hyperresponsiveness based on dose-response curves relating FEV1 to methacholine concentration. The normal subject is characterized by bronchoconstriction to methacholine only at high doses, with a 20% fall in FEV1 occurring above 64 mg/ml and a plateau at higher levels. The mild asthmatic is hyperresponsive to methacholine, as seen by the upward (increased response) and leftward (increased sensitivity) shift of the dose-response curve, resulting in a PC20 of 4 mg/ml, and no clear plateau. The severe asthmatic has even more hyperresponsiveness, with a further shift up and to the left of the dose response curve resulting in a PC20 of 1 mg/ml and no plateau. From [190], with permission.

Figure 15 – Pathways involved in direct and indirect airways hyperresponsiveness. On the left is depicted the events occurring in exercise or hyperpnea-induced bronchoconstriction, where respiratory water loss results in increased osmolarity of the airway surface liquid, which stimulates mediator release from mast cells and eosinophils, resulting in airway smooth muscle (ASM) constriction. On the right are the events involved in allergen challenge, where the allergen-IgE complex activates cellular inflammation and subsequent ASM constriction. Both exercise/hyperpnea and allergen challenges (or hypertonic saline, mannitol or adenosine monophosphate) are considered indirect challenges because they stimulate ASM constriction indirectly via proximal mediators and events. This is in contrast to methacholine or histamine challenge which result in direct stimulation of ASM. From [190], with permission.

Figure 16 – Mechanisms involved in airways hyperresponsiveness. Multiple factors govern airway responsiveness, including ASM contractility, mechanical load on ASM (opposing or enhancing ASM constriction), geometry of the airway (amplifying ASM constriction), and delivery of the agonist (enhancing the sensitivity to agonist). In addition, not shown directly, heterogeneity of airway constriction throughout the lung also contributes to responsiveness. From [12], with permission.

Figure 17 – Summary illustration of influence of altered lung mechanics on clinical manifestations of asthma. This highly schematic summary reminds us that many aspects of lung mechanics are involved in the clinical manifestations of asthma as measured by asthma severity and asthma control. These include increased airway resistance due to multiple factors governing airway caliber, including airway smooth muscle tone, airway wall thickness and stiffness,

surrounding lung elastic recoil and airway-parenchymal interdependence. Subsequent effects include airflow limitation, gas trapping, ventilation heterogeneity, and airway hyperresponsiveness. Adapted from [192], with permission.

Didactic Figure Legends

Figure 1

Teaching Points: Multiple inflammatory pathways are implicated in the pathogenesis of asthma, as illustrated by this summary figure. The healthy airway is depicted at the top, with airways smooth muscle surrounding the airway epithelium sitting on the reticular basement membrane. Moving counterclockwise, eosinophilic asthma is seen by increased eosinophilic inflammation driven by allergen stimulation resulting in increased eosinophilic activation by IL4, 5, and 13, and mast cell activation by IgE via TH2 cells. Next is seen non-allergic eosinophilic inflammation driven by pollutants and microbes resulting in increased eosinophilic inflammation activation by IL5 via ILC2 cells driven by IL33, TSLP and PGD2. In the lower right section is seen Type 1 and Type 17 neutrophilic inflammation, driven by pollutants, microbes and oxidative stress, resulting in increased neutrophil activation by CXCL8 via macrophage and TH1 and TH17 cells. Non-eosinophilic asthma may also be paucigranular (top right), with few inflammatory cells, or mixed granulocytic, with both eosinophilic and neutrophilic inflammation (bottom). From [195], with permission.

Figure 2

Teaching Points: Multiple factors determine airway narrowing, as shown in this diagram. Airway caliber is regulated by surrounding airway smooth muscle force balanced against parenchymal tethering. Other factors that modulate airway caliber include the thickness of the airway wall due to inflammation, edema and remodeling, mucosal folding, and the elastic properties of the airway wall. From [97], with permission.

Figure 3

Teaching Points: The effects of a deep inhalation (DI) can be described by considering the relative hysteresis of airway and lung parenchyma. Hysteresis is reflected in the area inscribed by the inspiratory and expiratory pressure (P) vs. volume (V) curves. An increase in airway hysteresis means that a dilated airway retains a more relaxed state after inspiration. An increase in parenchymal hysteresis means that after the lung parenchyma is stretched, it exerts less of a recoil pressure on embedded airways. Accordingly, 3 states are shown. In A), airway and parenchymal hysteresis are equal, so that a deep inhalation results in no change in airway caliber, as seen by the symmetrical balance of Pressure for a given Volume on the left, and the equal diameters of the airways on the right. In B), PV curves showing airway greater than parenchymal hysteresis, which results in a bronchodilator response to deep inhalation, as seen by the greater dilator force after inhalation. C) PV curves showing parenchymal greater than airway hysteresis, which results in a bronchoconstrictor response to deep inhalation, as seen by the greater constrictor force after inhalation. From [207], with permission.

Figure 4

Teaching Points: Peak flow can vary greatly over time, as shown in this example recording of peak flow measured twice daily over 150 days. The statistical pattern of fluctuation is similar at different time scales, as seen by the inset showing magnification of a shorter time period, illustrating the fractal properties of this time series, meaning that the pattern fluctuates with a similar pattern no matter the amount of time considered (e.g., minutes to hours to days). From [75], with permission.

Figure 5

Teaching Points: This tracing of a Single Breath Nitrogen Washout from a healthy 21 year old male without respiratory disease illustrates multiple points about gas distribution within the lung. The participant has inhaled 100% O2 from residual volume (RV) to total lung capacity (TLC) and then expired from TLC to RV. The expiratory nitrogen trace is comprised of four distinct phases: phase I (dead space gas containing pure oxygen from the inhalation), phase II (bronchial ventilation showing sudden rise in nitrogen coming from conducting airways that received little oxygen during inhalation), phase III (alveolar ventilation representing the net sum of nitrogen concentrations coming from all the alveolar spaces as the lung progressively empties) and phase IV (sudden increase in nitrogen concentration indicating onset of airway closure where only the most distal alveoli empty their nitrogen content). Closing volume (CV, blue bar) is the expired volume between the onset phase IV and RV. Closing capacity (CC) is calculated as CV + RV. The slope of phase III is plotted between 25% and 75% of vital capacity (orange dashed line) and reflects the eveneness of ventilation; the flatter the slope, the more homogeneous is overall ventiation. Note the unconventional x-axis in which volume been plotted based on total lung capacity measured by body plethysmography to show CC and RV.

Figure 6

Teaching Points: This figure illustrates the effects of homogeneous vs. heterogeneous airway constriction on Resistance and Elastance measured by the forced oscillation technique. Left, resistance is plotted against frequency and compared between the healthy state and different states of airway constriction. Under conditions of homogeneous constriction, there is relatively uniform elevation of R across the frequency range. Under conditions of heterogeneous

constriction, there is marked frequency dependence, with R at lower frequencies being markedly elevated. Right, effects of different airway constriction patterns on elastance (E) relative to the healthy state. Homogeneous constriction causes most of the oscillatory input signal to be shunted into the central airways because it cannot penetrate the homogeneously constricted peripheral airways, resulting in a uniform increase of E, which rises with frequency because higher frequency flow signals can penetrate less and less into the periphery. Heterogeneous airway constriction results in a marked rise in E with a jump in E at low frequencies around the breathing frequency reflecting airway closure. Note that the rise in E in both circumstances does not necessarily reflect a pure stiffening of the lung parenchyma, but rather is related to the pattern and degree of rise in R; in this way, it is a functional increase in lung stiffness, rather than related to stiffer structural elements. From [156], with permission.

Figure 7

Teaching Points: This tracing illustrates the process of lung de-recruitment measured by forced oscillation technique in a 21 year old healthy male without respiratory disease. The participant performed a slow vital capacity during which respiratory system reactance (Xrs) was continuously measured and the points of de-recruitment (DR1 = diamond, DR2 = circle) calculated as previously described[185]. Essentially, as the lung empties, and de-recruitment results in airway closure, there are abrupt increases in E (similar to as described in Figure 6) that are seen by abrupt more negative measures of Xrs. Note that functional residual capacity (FRC) occurs above both de-recruitment points and that closing capacity (CC) measured from the Single Breath Nitrogen Washout test occurs at approximately the same volume as DR2.

Figure 8

Teaching Points: This is an example of imaging of heterogeneous ventilation by hyperpolarized helium MRI. Shown in this example are the ventilation images obtained from a healthy subject (A), compared to 3 different subjects with mild (B), moderate (C) and severe (D) asthma. Notice the increasing number of ventilation defects (white arrows), reflecting functional airway closure, as asthma becomes more severe. From [225], with permission.

Figure 9

Teaching Points: This is an example of imaging of heterogeneous ventilation by ventilation SPECT/CT. Shown are the images from one subject before (left) and after (right) methacholine. Not only are there larger and new poorly ventilated or non-ventilated spaces (black), but the ventilation has become more heterogeneous within the ventilated airspaces, as seen by the color-coding of ventilation and the histogram distribution of ventilation below the images. From [70], with permission.

Figure 10

Teaching Points: This figure illustrates the nitrogen trace from a Multiple Breath Nitrogen Washout test. The participant was instructed to take tidal volume breaths of approximately 1 liter until end-expiratory nitrogen concentration had fallen by $1/40^{th}$ of the initial concentration (~2.5%). A measure of ventilation heterogeneity, the Lung Clearance Index (LCI) is calculated as the lung turnover (cumulative expired volume/functional residual capacity) at this point, meaning, how many FRC-sized volumes did it take to washout the nitrogen to this pre-defined level. Insets show the phase III slope *normalized* by the mean expiratory nitrogen concentration

for the 1st and 25th breaths, respectively, which is done so that the slopes at each point may be compared to each other.

Figure 11

Teaching Points: This tracing illustrates the derivation of two parameters of ventilation heterogeneity from the Multiple Breath Nitrogen Washout test. The slope of the plot between lung turnovers 1.5 and 6 reflects ventilation heterogeneity occurring in airways where gas transport is dependent upon convection. This slope is termed Scond as it is felt to represent ventilation at the level of the peripheral conducting airways. The (normalized) phase III slope of the first breath (minus the contribution of Scond; i.e., Scond x lung turnover at first breath) reflects ventilation heterogeneity at the interface between convection and diffusion gas transport, known as the diffusion front. This is felt to represent the heterogeneity of the ventilation at the entrance to the acinar region, and is termed Sacin. Data are shown from a 34 year old male with normal lung function (△) and a 45 year old female with asthma (○). The data from the asthmatic person reveals higher Scond and Sacin, indicating more ventilation heterogeneity in both regions, which is associated with asthma.

Figure 12

Teaching Points: This is a conceptual illustration of the anatomic areas of involvement in determining conduction-dependent and diffusive-conductive dependent ventilation. While not meant to precisely indicate anatomic location, Scond is seen to arise in more proximal conducting airways, and Sacin is seen to arise in more distal airways at the junction between conductive and acinar ventilation. From [271], with permission.

Figure 13

Teaching Points: This illustration is a conceptualized model of how parallel and serial airway interdependence leads to heterogeneous ventilation. Shown is a symmetrically bifurcating airway tree under uniform ASM constriction (red lightning bolts). On the left is illustrated the consequences of parallel interdependence of airways on ventilation. One airway has a slightly thicker airway wall than the other (red area),which may be related to airway inflammation, or remodeling. Accordingly, during ASM constriction, airflow is reduced more to this airway than its daughter branching airway (dashed arrow). The reduced airflow leads to reduced distal ventilation, and hence reduced expansion of the surrounding lung parenchyma. This leads to reduced tethering forces on the embedded airway, allowing less stretch of the ASM, and therefore enhancing its constriction. This pattern results in a vicious cycle (curving arrows) culminating in widespread loss of ventilation to the parenchyma fed by that airway ("catastrophic airway collapse"). This is seen as a ventilation defect seen on imaging (Vdef). Meanwhile, under conditions of conserved total minute ventilation, airflow is diverted away from the main airway into the daughter airway (slightly thick arrow) without airway wall thickening increasing ventilation to the parenchyma served by that airway. This results in increased expansion of that parenchymal area with consequent increased tethering forces and increased tidal ASM stretch and dilation of the airway. This process also cycles until a dynamic equilibrium of airway size and parenchymal expansion is reached.

On the right is shown the consequences of serial interdependence of airways. Transmural pressure across the airway is greater for central than peripheral airways during tidal breathing,

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Teaching Points: This figure illustrates pathways involved in direct and indirect airways hyperresponsiveness. On the left is depicted the events occurring in exercise or hyperpnea-induced bronchoconstriction, where respiratory water loss results in increased osmolarity of the airway surface liquid, which stimulates mediator release from mast cells and eosinophils, resulting in airway smooth muscle (ASM) constriction. On the right are the events involved in allergen challenge, where the allergen-IgE complex activates cellular inflammation and

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Figure 17

Teaching Points: This highly schematic summary reminds us that many aspects of lung mechanics are involved in the clinical manifestations of asthma as measured by asthma severity and asthma control. These include increased airway resistance due to multiple factors governing airway caliber, including airway smooth muscle tone, airway wall thickness and stiffness, surrounding lung elastic recoil and airway-parenchymal interdependence. Subsequent effects include airflow limitation, gas trapping, ventilation heterogeneity, and airway hyperresponsiveness. Adapted from [192], with permission.

Further Reading

Textbooks:

Barnes PJ, Grunstein MM, Leff AR, Woolcock AJ, Eds. Asthma. New York: Lippincott-Raven Publishers, 1997.

Bates JHT. Lung Mechanics. New York: Cambridge University Press, 2009.

Crystal RG, Barnes PJ, West JN, Weibel ER, Eds. The Lung: Scientific Foundations. New York: Lippincott-Raven Publishers, 1997.

Cross-References

Airway-Parenchymal Interdependence

Complexity and Emergent Phenomena

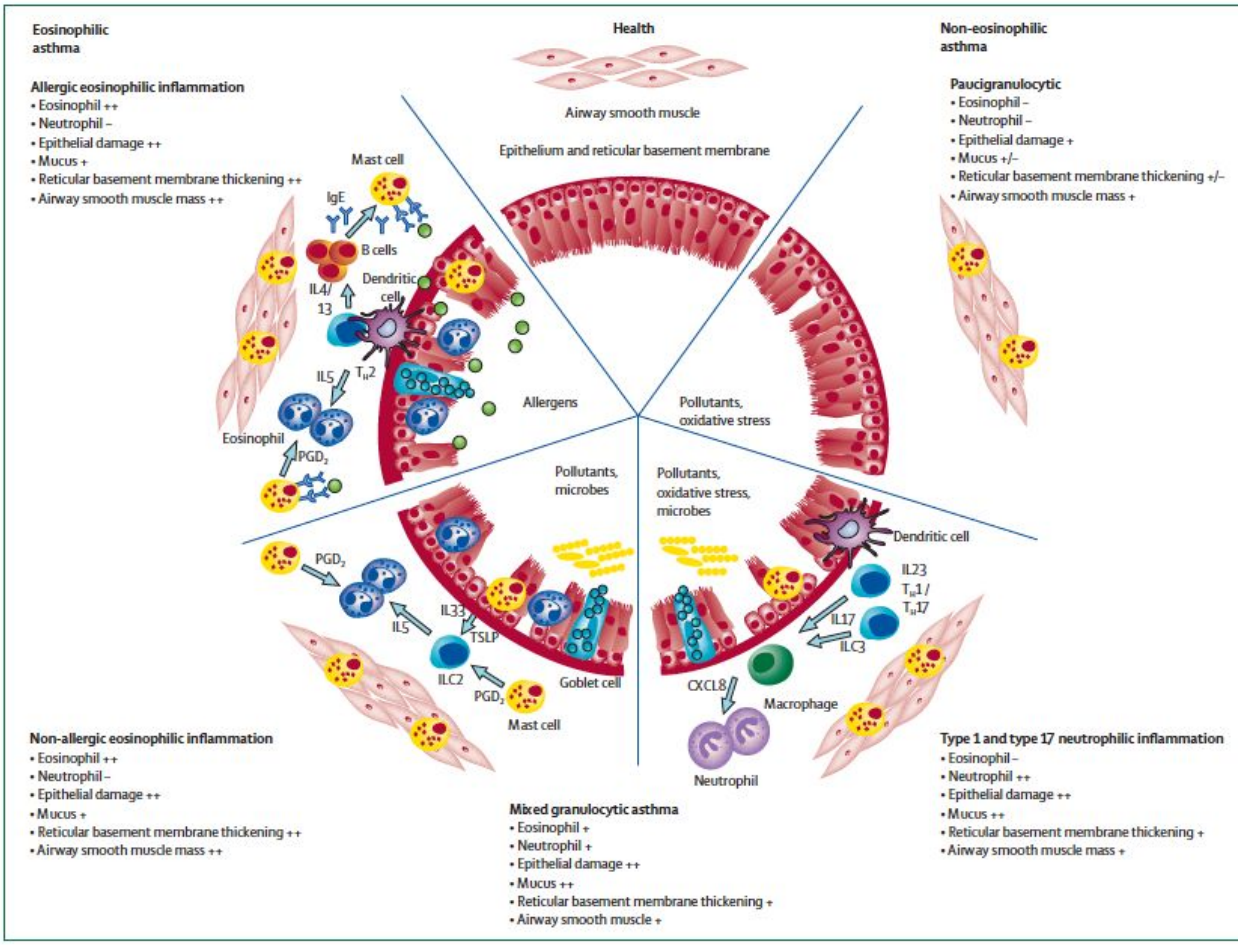
Distribution of Ventilation

Expiratory Flow Limitation

Pathophysiology of Asthma

Figures

Figure 1



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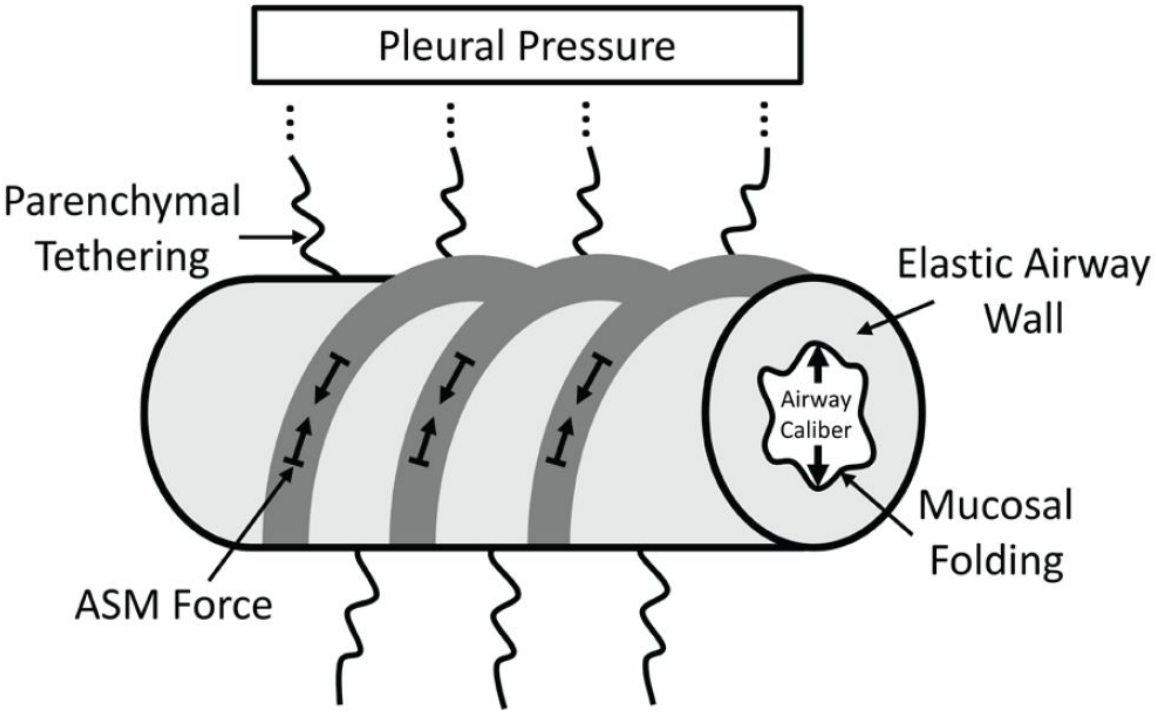


Figure 3

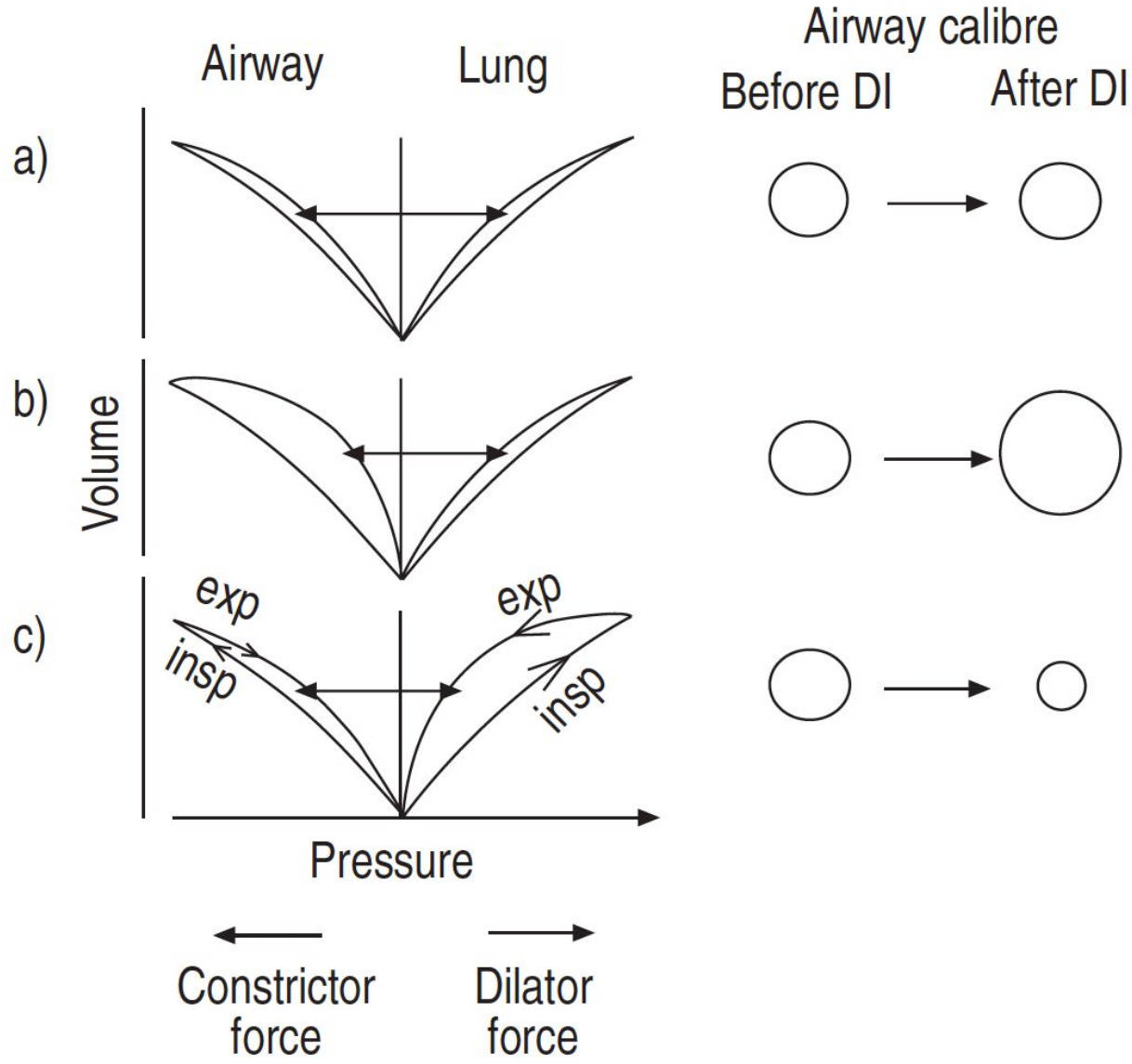
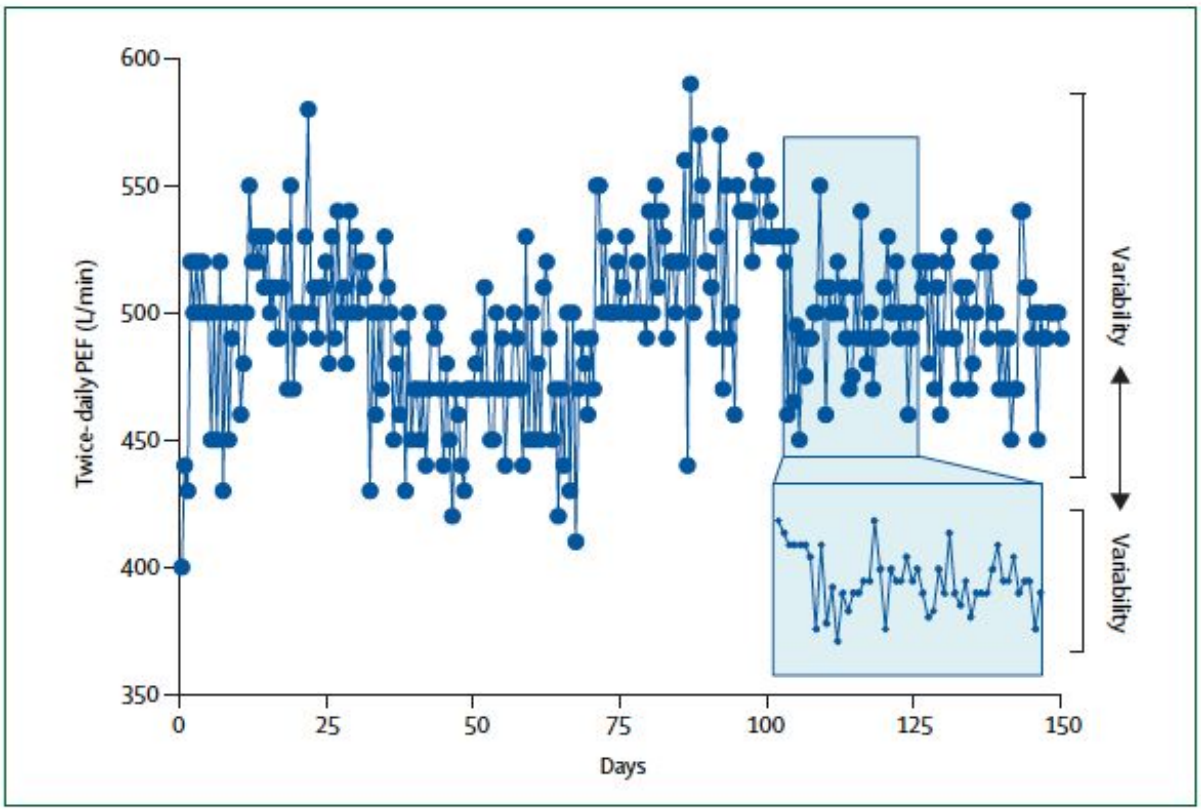

Airway
Lung
a)
b)
c)
Volume
exp
insp
exp
insp
Pressure
Constrictor force
Dilator force
Airway calibre
Before DI
After DI

Figure 4



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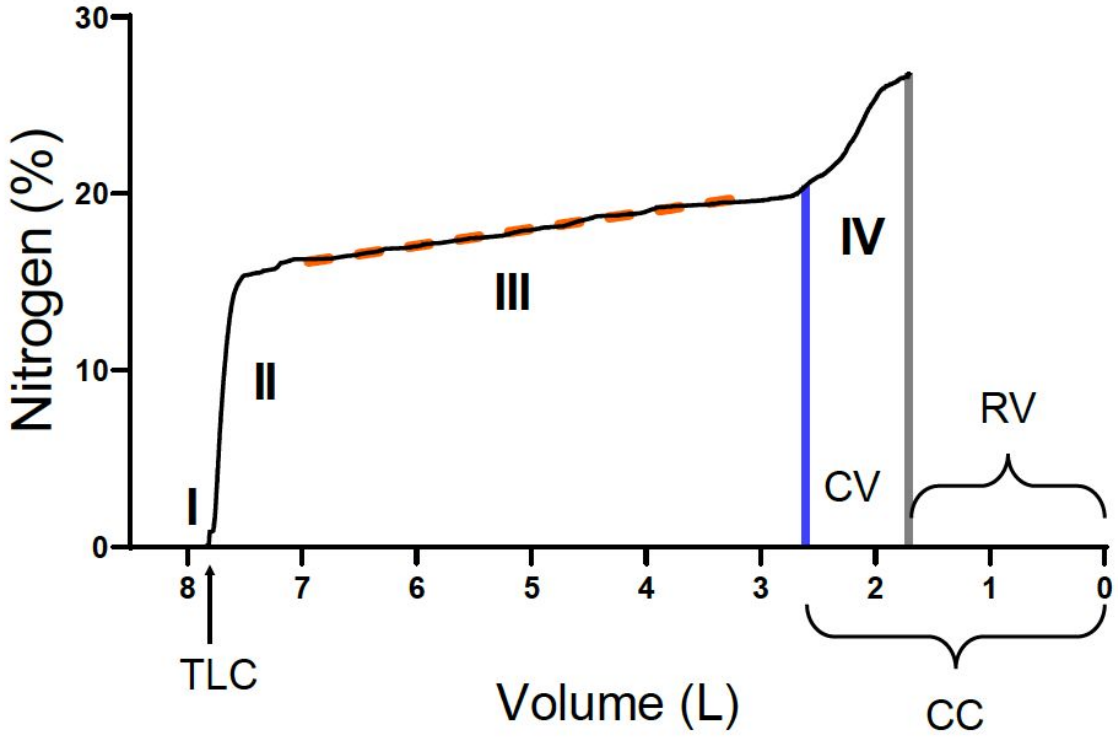

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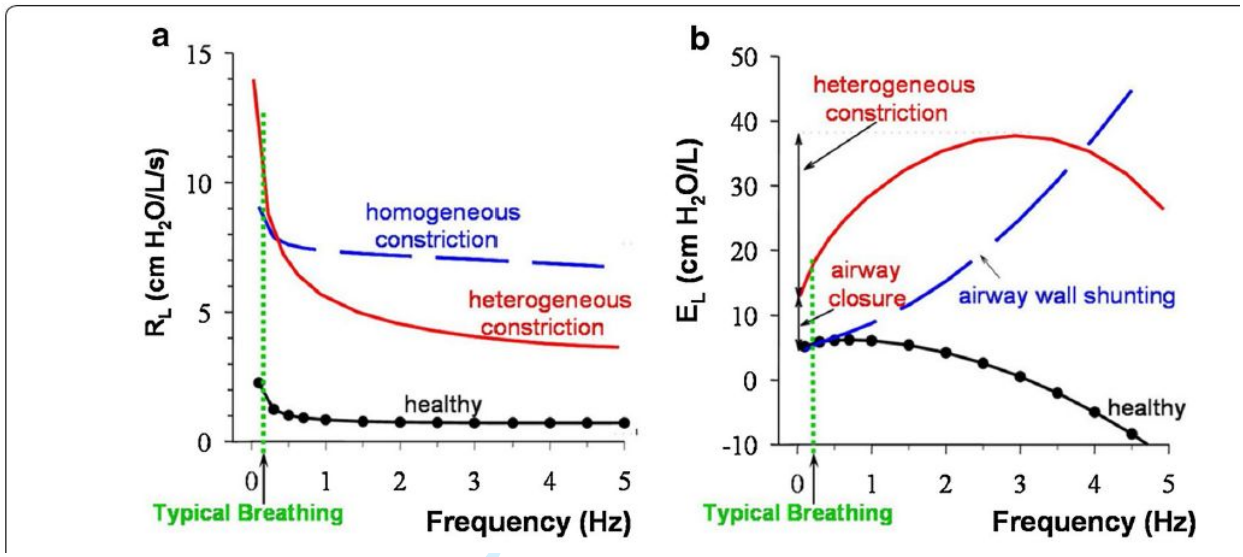

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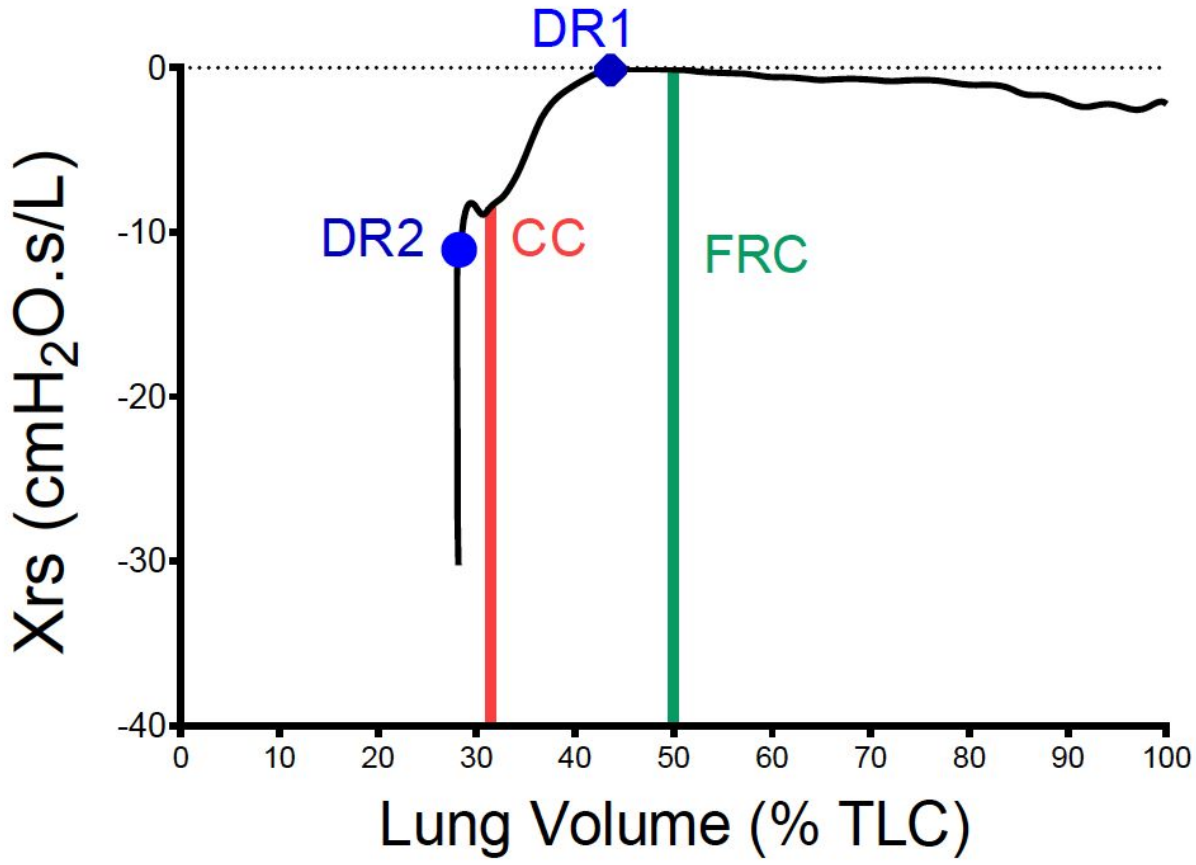

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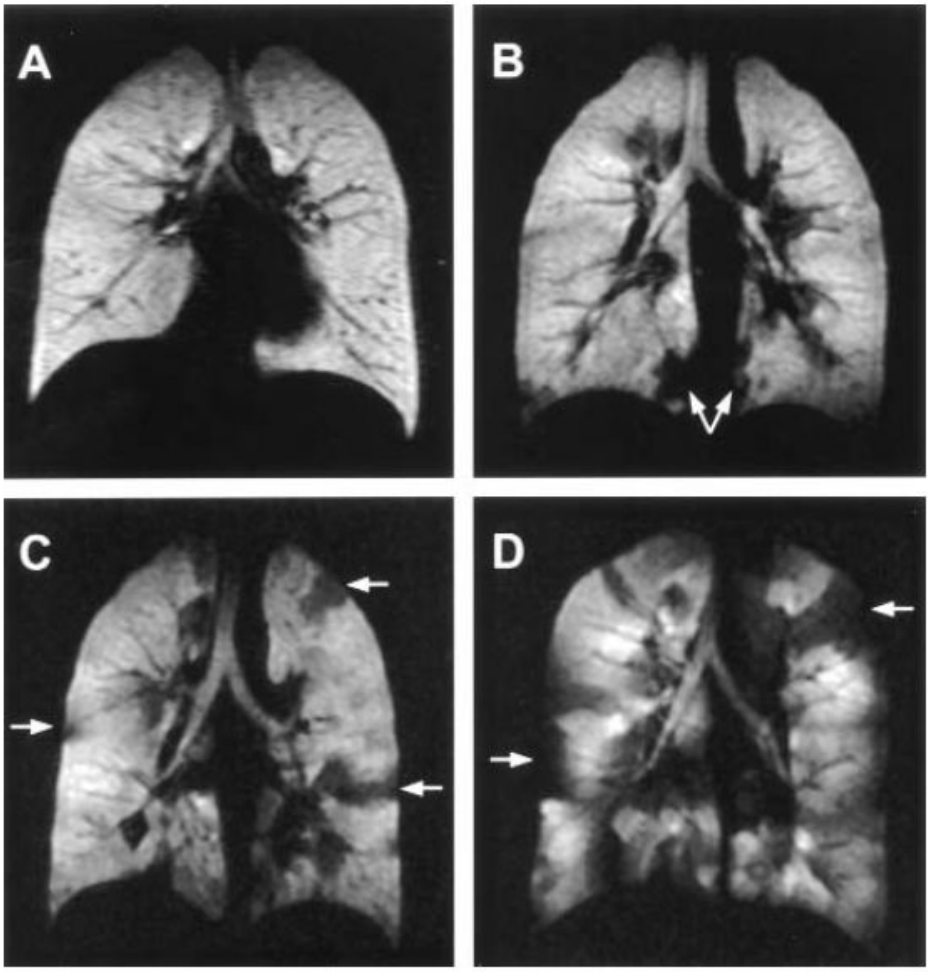
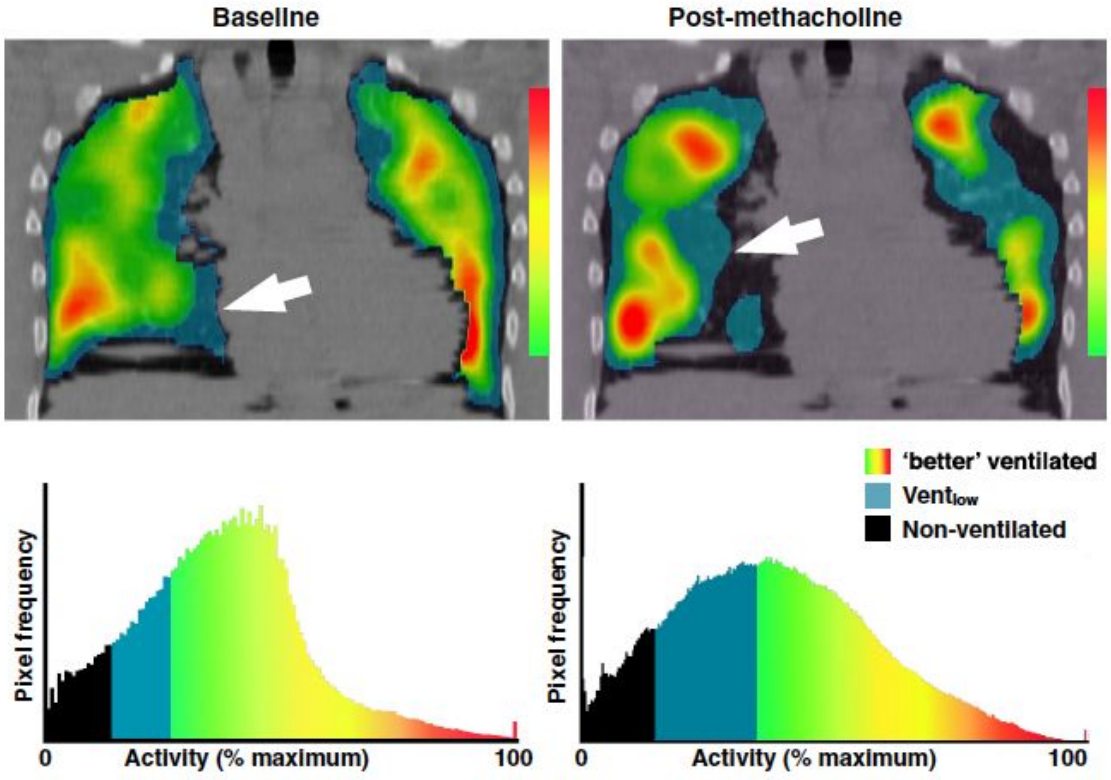


Figure 9



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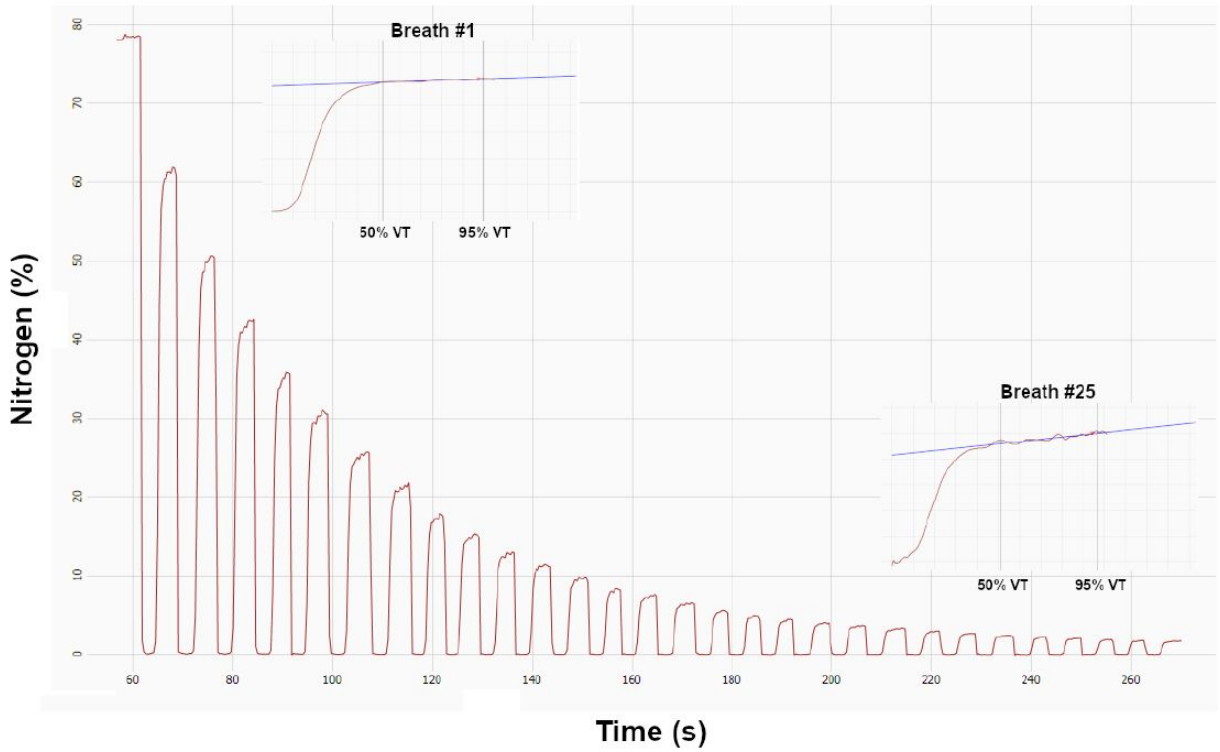

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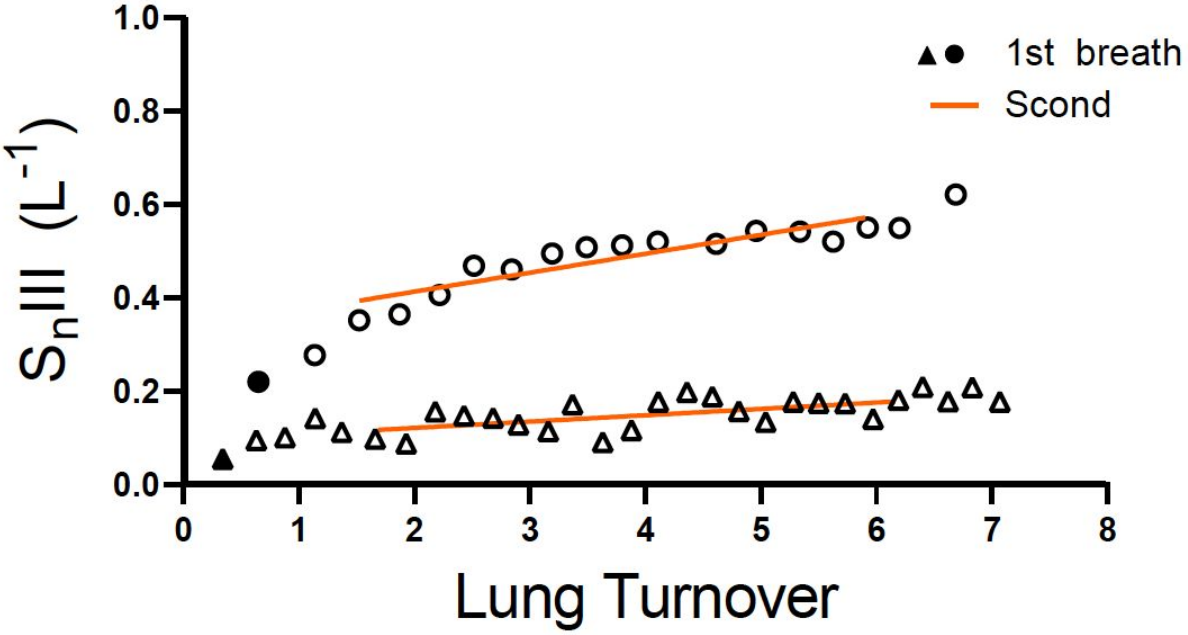
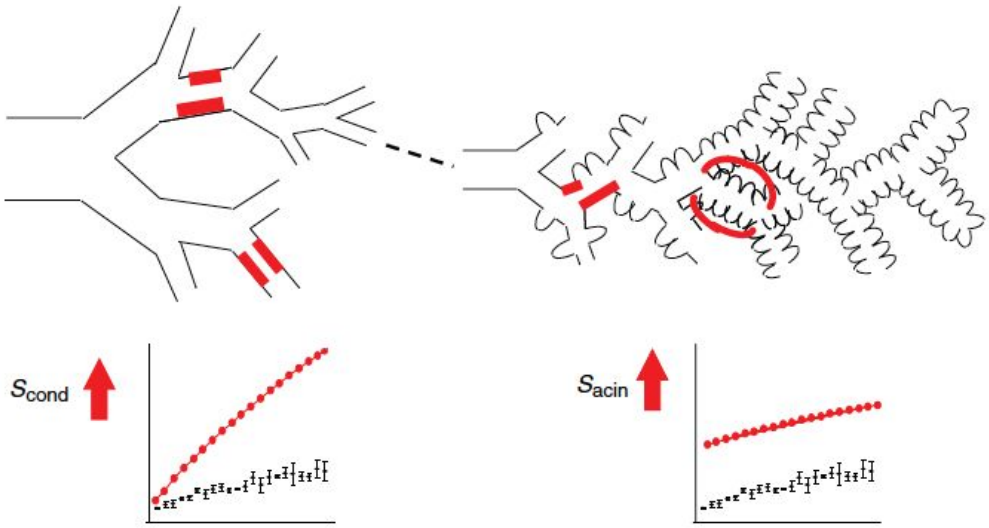


Figure 12



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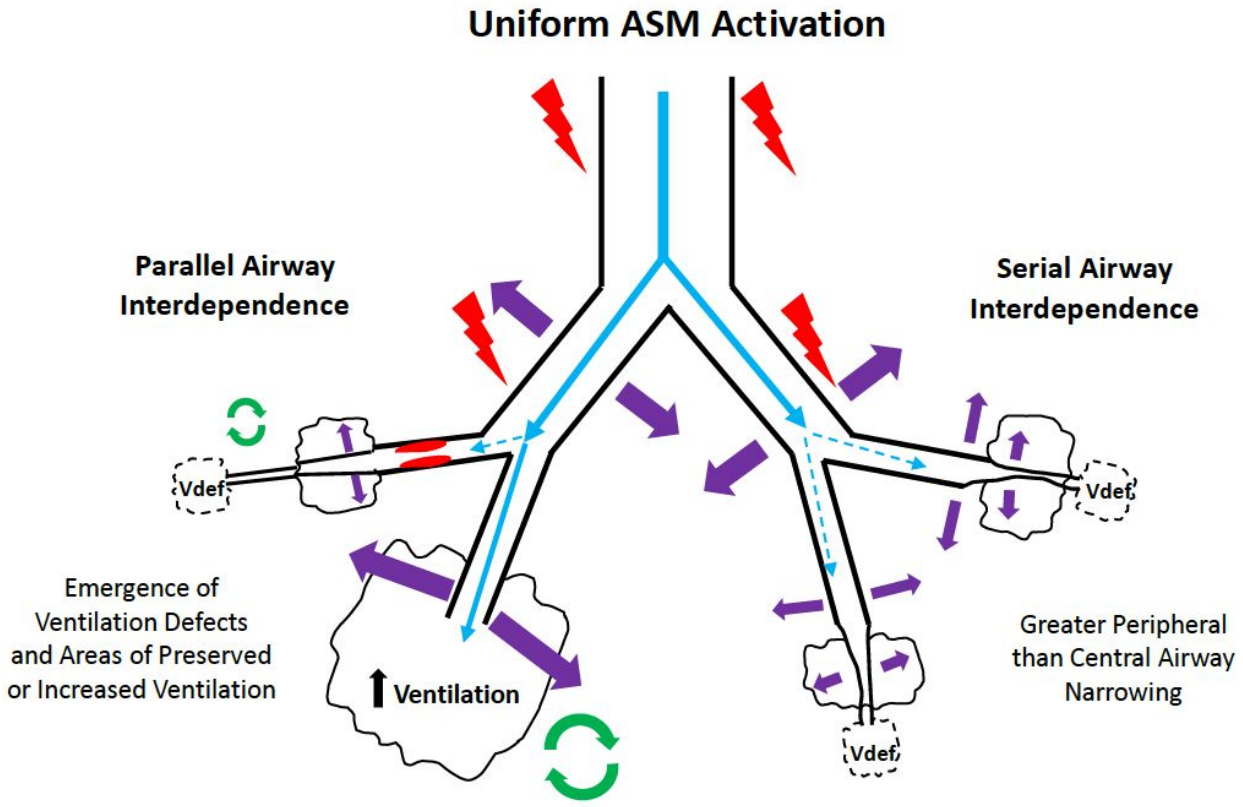

Uniform ASM Activation
Parallel Airway Interdependence
Serial Airway Interdependence
Vdef
Vdef
Emergence of Ventilation Defects and Areas of Preserved or Increased Ventilation
Ventilation
Vdef
Greater Peripheral than Central Airway Narrowing

Figure 14

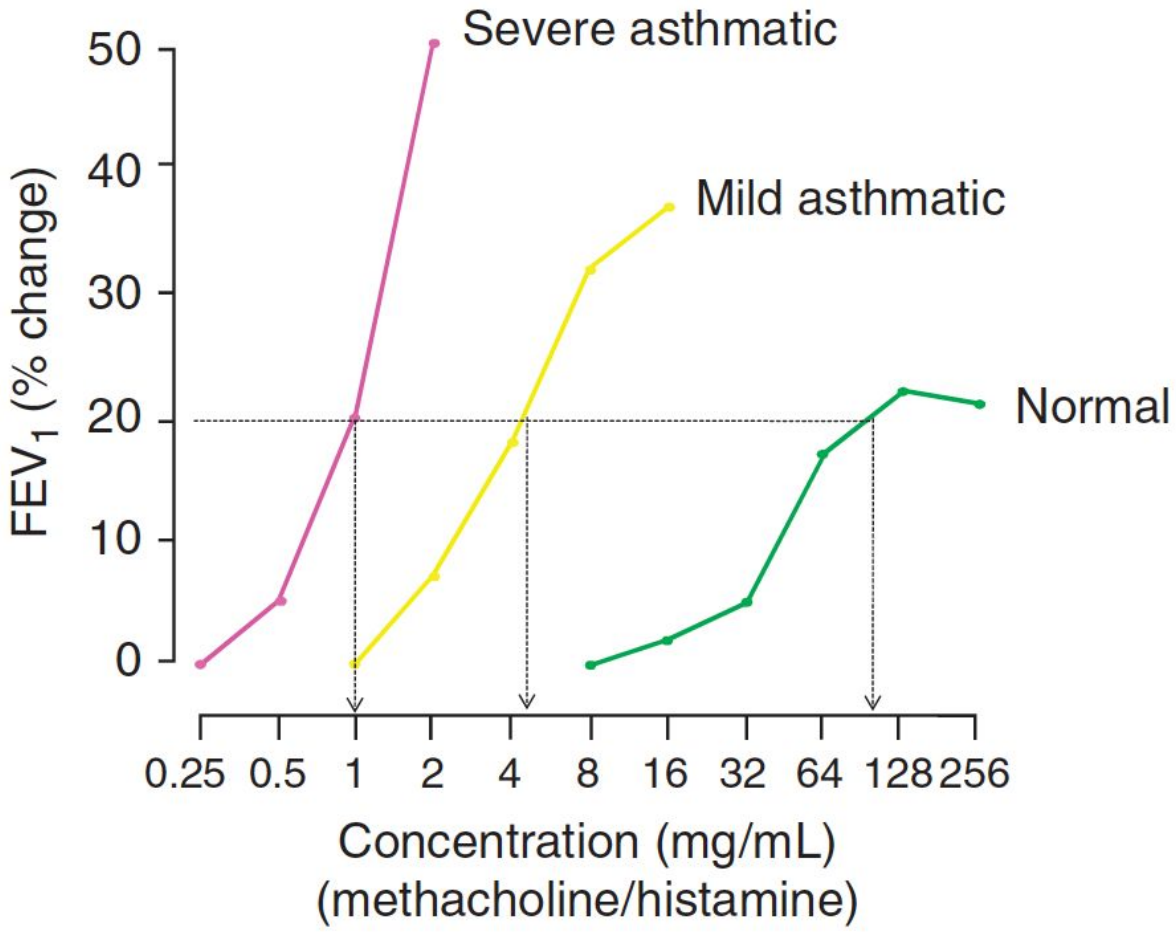

Severe asthmatic
Mild asthmatic
Normal
FEV1 (% change)
50
40
30
20
10
0
0.25 0.5 1 2 4 8 16 32 64 128 256
Concentration (mg/mL)
(methacholine/histamine)

Figure 15

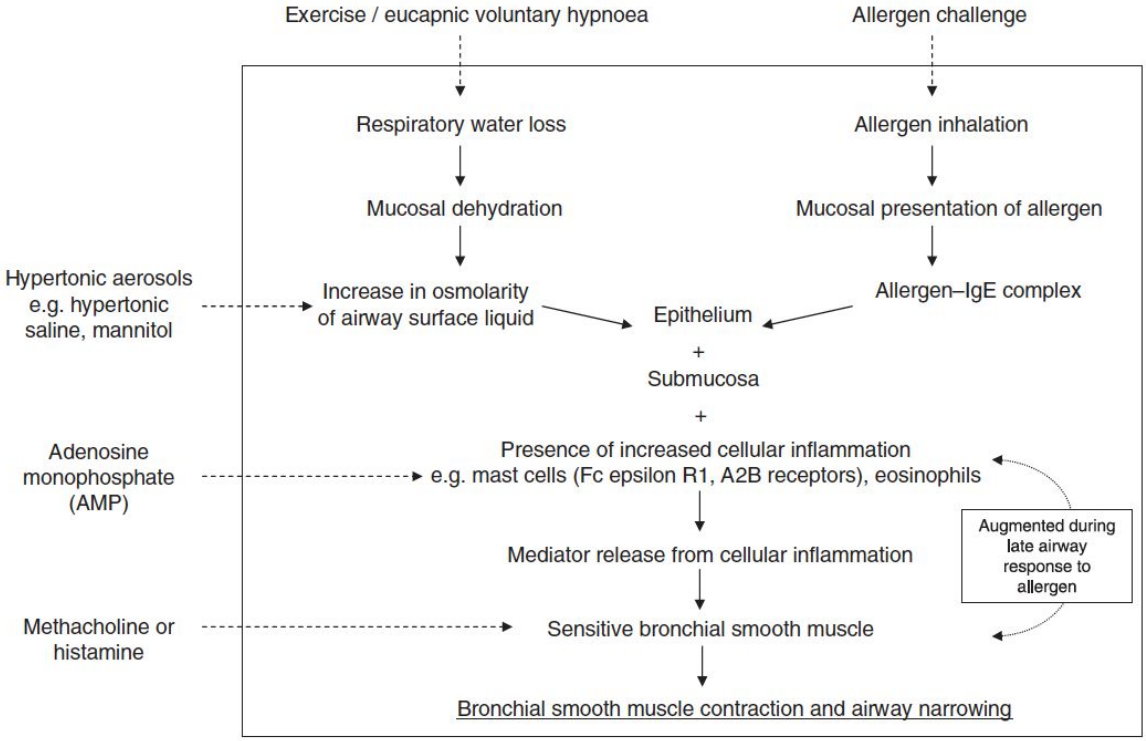
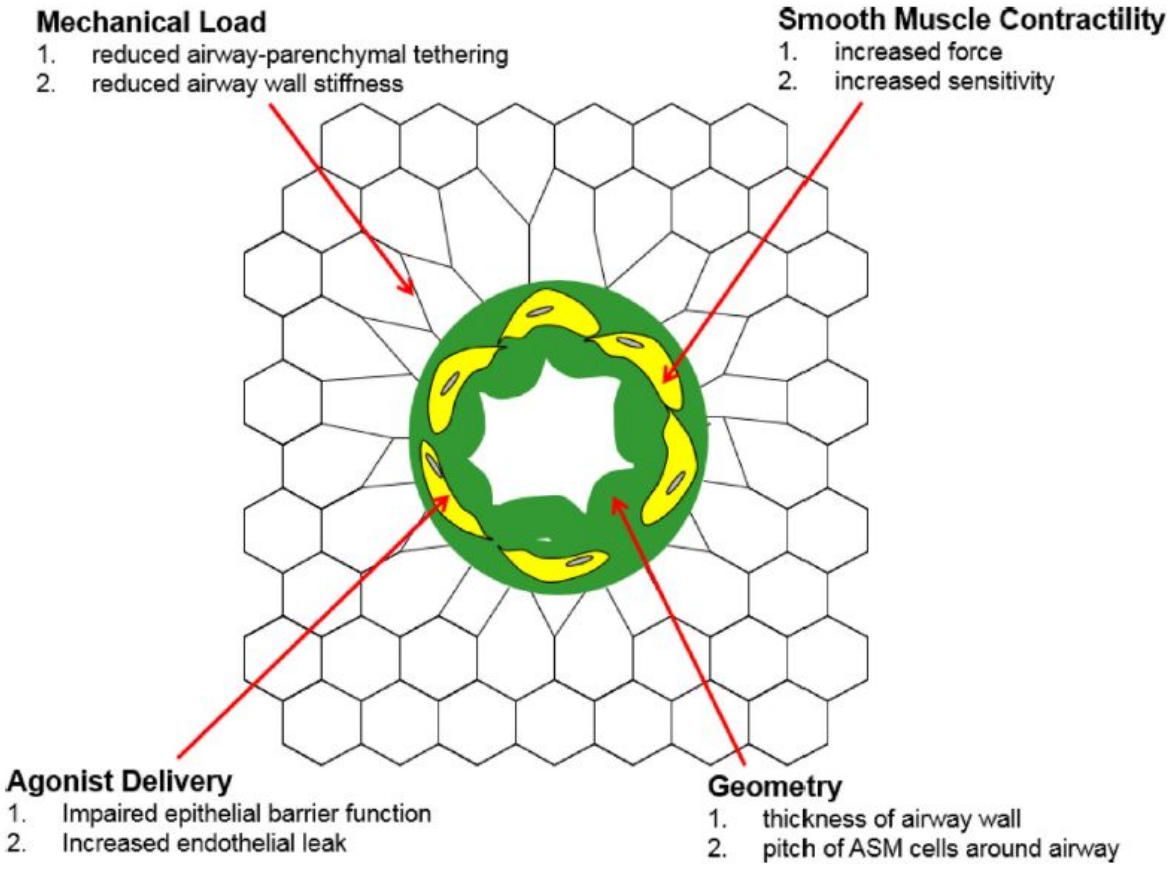


Figure 16



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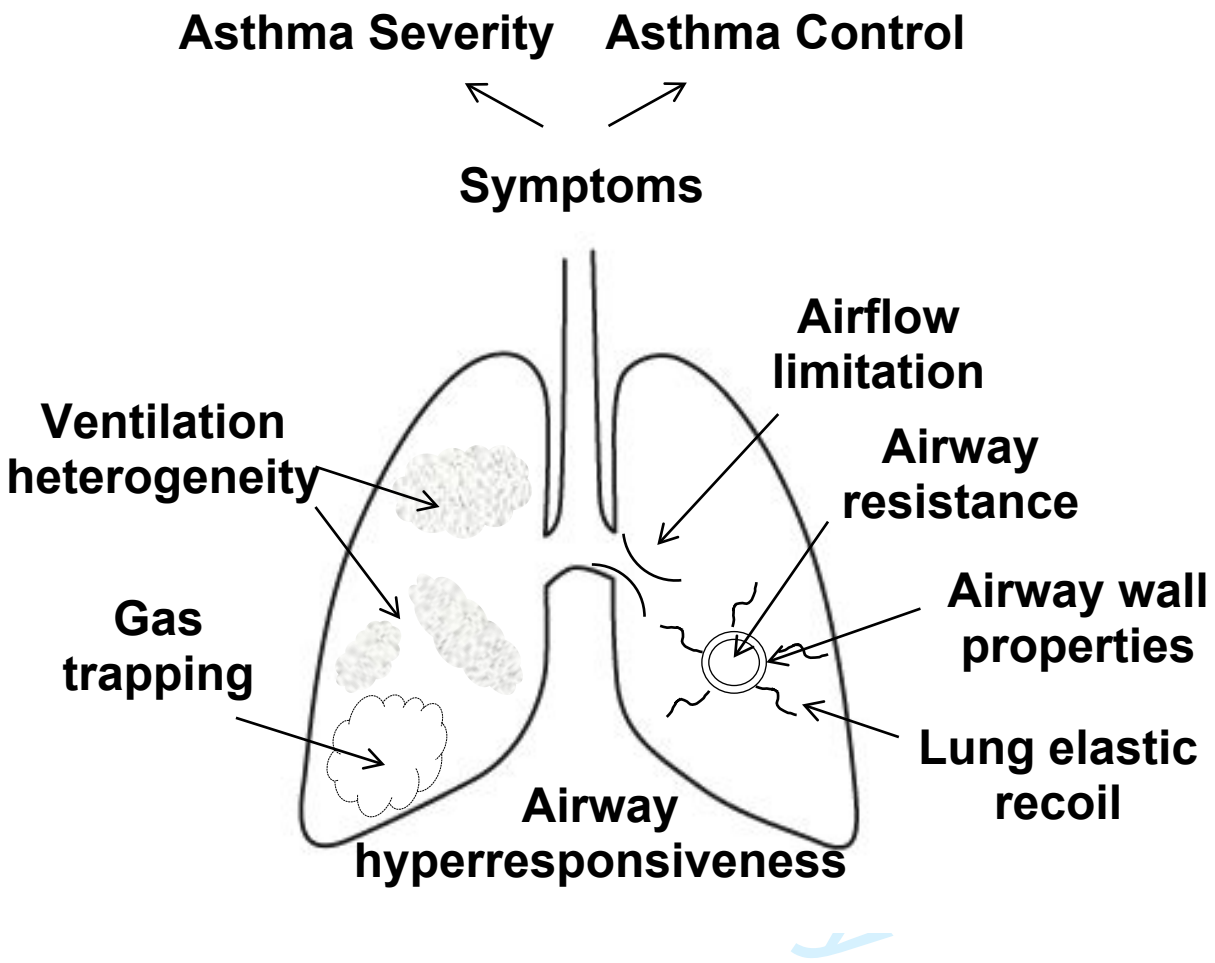
Asthma Severity
Asthma Control
Symptoms
Airflow limitation
Ventilation heterogeneity
Airway resistance
Airway wall properties
Gas trapping
Lung elastic recoil
Airway hyperresponsiveness