Towards restoring the physiological protection against airway narrowing in asthma : take a deep breath!

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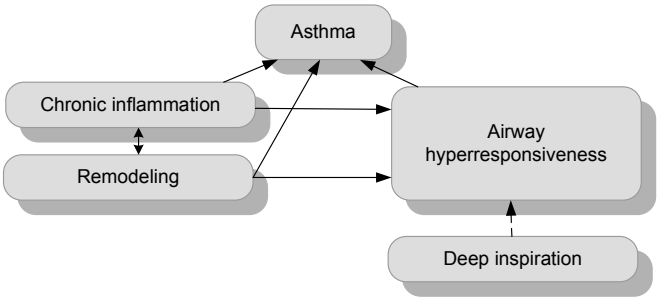
Chapter 1

General introduction

1. Introduction

Asthma is defined as a chronic inflammatory disorder of the airways, associated with an increase in airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, and cough[1]. Airway hyperresponsiveness is a term used to indicate that the airways narrow too easily and too much in response to different provoking stimuli, such as allergens or cigarette smoke. Although in this definition inflammation and hyperresponsiveness are causally related, the mechanism underlying this association has not been elucidated. Deep inspirations affect airway narrowing, and may therefore play a role in airway hyperresponsiveness in asthma **(Figure 1)**.



1.1. Chronic airway inflammation and remodeling in asthma

The role of airway inflammation in asthma has been established by findings in resection material in patients with fatal asthma[2-4], but also from analyses of bronchial biopsies of patients with milder forms of asthma[5]. These inflammatory changes were found throughout the central and peripheral airways[6-8], and often vary with the severity of the disease[9]. The changes observed can be divided into inflammatory cell infiltration in the airway wall and structural changes of the airway wall in response to, or in parallel with, chronic inflammation (remodeling).

1.1.1. Inflammatory cells

Cell types that have been identified as characteristic for asthmatic inflammation are degranulated mast cells, eosinophils and lymphocytes. More recently, infiltration of the smooth muscle bundles by mast cells have been found to be characteristic for asthma as well[10,11]. *Mast cells* release histamine in response to IgE binding to the high-affinity receptor on the cell. Histamine induces smooth muscle contraction leading to airway narrowing. *Eosinophils* are a source of inflammatory proteins[6,9], including the major basic protein, that can damage the epithelium, degranulate mast cells and intensify bronchial hyperresponsiveness. The eosinophil is also a rich source of leukotrienes that contract smooth muscle and increase vascular permeability[12]. *T lymphocytes* may play a role in asthmatic inflammation by the release of cytokines. There are two types of CD4+ T helper(h) cells. Type 1 Th cells produce interleukin (IL)-2 and interferon

(IFN)-γ, which are essential for cellular defense mechanisms. Type Th 2 cells produce cytokines (IL-4, 5,6,9 and 13) that mediate allergic inflammation[13,14]. Observations in animal models and patients with asthma[15] suggest that allergic inflammation in asthma results from a Type 2 mediated mechanism.

Inflammatory cells and mediators may thus influence the response of airway smooth muscle to direct or indirect stimuli, by either directly activating the smooth muscle cells or inducing a cytokine and chemokine driven pathway that mediates both inflammation and smooth muscle function.

1.1.2. Structural changes

Changes in the structural components of the airway wall are often referred to as the remodeling process. Characteristic observations of structural changes are epithelial disruption, thickening of the basal membrane (collagen deposition beneath the basal membrane), hyperplasia and hypertrophy of airway smooth muscle[16-18], changes in extracellular matrix in- and outside the airway smooth muscle bundles[3,19], increased number of vessels and vascular permeability, and hyperplasia of goblet cells.

In principal, remodeling is thus a repair mechanism. However, in asthma it may be inappropriate since it alters the structural properties of the airway wall in a way that it affects the physiology of airway mechanics:

- Epithelial disruption may lead to an easy access of stimuli to the smooth muscle cells, and is related to airway hyperreactivity[20]
- Thickening of the basal membrane may provide load against smooth muscle contraction by stiffening the airway wall, or favors airway closure by preventing mucosal foldings[21-24].
- Hyperplasia or hypertrophy of airway smooth muscle may increase force generation and shortening capacity[25,26].
- Increased vascular permeability may lead to airway wall edema that thickens the airway wall and with that reduce the lumen and increases the outer perimeter. It may also lead to stiffening of the airway wall.
- Deposition of extracellular matrix may increase the load on airway smooth muscle contraction when situated on the inside of the smooth muscle layer[27,28], but may also reduce the parenchymal load when present in the outer layer of the airway wall.
- Collagen deposition within the airway smooth muscle bundles may modulate force transference among the contractile cells[24,29].

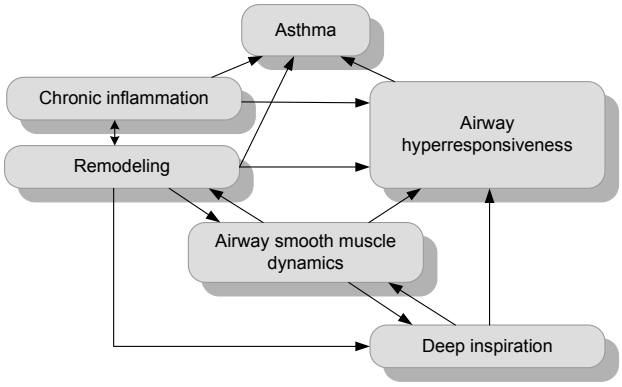
Therefore, not only acute and chronic inflammatory processes may lead to airway hyperresponsiveness, also the structural changes alter the mechanical properties of the airway wall and thus play a large role in the net effect of airway smooth muscle contraction.

1.2. Airway hyperresponsiveness

Individuals without asthma respond less to non-specific contractile agonists. This is reflected by a maximal response plateau on a dose-response curve with only modest airway narrowing at the highest dose[30-32]. In patients with asthma the plateau is elevated or completely abolished, indicating excessive airway narrowing. Also the dose-response curve is often shifted to the left, indicating a higher sensitivity to the contractile agent.

The parenchyma provides an elastic load against which the smooth muscle must shorten. The elastic recoil pressure is lung volume dependent, meaning that the elastic load presented to the airway smooth muscle increases as lung volume increases[33]. And vice versa the maximal response to a given agonist decreases as lung volume increases[34].This suggests that elastic load on airway smooth muscle is the principal determinant of maximal airway smooth muscle shortening in normal subjects. Although this load is appreciable it may not be sufficient to explain the plateau observed in healthy subjects. If the airways are sufficiently stimulated with contractile agonists, complete closure of even large cartilaginous conducting airways can readily occur within the lung at FRC[35]. Thus the elastic load of the parenchyma is not enough to prevent full airway closure at a static low lung volume. What other determinant of lung volume could then explain the protective mechanism of maximal response? It is speculated that the target of airway modulation by lung volume change is the airway smooth muscle cell itself[36,37], leading to less contractility[38]. Changes in the mechanism that modulates smooth muscle function by lung volume changes, can therefore indirectly change airway responsiveness to contractor stimuli. Therefore, it appears that the dynamic environment in the lung, as a result of continuously stretching forces during tidal breathing and occasional deep breaths, plays a major role in maintaining this environment in a condition where excessive airway narrowing is unfeasible or at least can be reversed by the system itself.

Taken together, in order to understand the pathophysiology of asthma one needs to clarify the role of stretch, i.e. deep inspirations, in the dynamic environment of the lung, and its interaction with airway smooth muscle, airway hyperresponsiveness, remodeling processes and chronic airway inflammation **(Figure 2)**.



2. Deep inspirations

2.1. History

Every subject takes a deep breath every 3 to 6 times an hour. This is usually interpreted as a sign of tiredness or boredom..., but they appear to have a physiological meaning as well. Already in 1859, the respiratory physician Henry Salter[39], observed that deep inspirations had different effects on the breathing pattern of his asthmatic patients:

"The spasm may be broken through, and the respiration for the time rendered perfectly free and easy, by taking a long, deep, full inspiration. In severe asthmatic breathing this cannot be done".

It was not until 1948 that Melville and Caplan[40] demonstrated in dogs that a maximal inflation of the lungs overcomes experimentally induced bronchoconstriction. In 1961 Nadel and Thierney[41] showed that in non-asthmatic humans deep inspirations also temporarily reduced airway narrowing. Since then, many studies have been performed to either examine the different effects of deep inspiration on airway narrowing in health and disease, but also to explore the pathophysiological mechanism of deep inspiration-induced bronchodilation.

2.2. Measuring airway responses to deep inspiration

Up till now, no standardized methodology has been described to measure airway responses to deep inspiration. Thus, many modified bronchoconstrictor challenges and different methods of lung function evaluation have been developed to do so[42]. The modified challenges are used to induce airways obstruction in healthy subjects, but also to measure airway responses to deep inspiration without previously performed deep inspirations[43,44]. Modified spirometry is used to compare flow rates on the expiratory flow-volume curve following a partial inhalation or a maximal inhalation (M/P ratios) and to observe the change in this ratio during a standard challenge[32,45]. Measurements of respiratory resistance by forced oscillation technique enables researchers to continuously measure resistance during tidal breathing and deep inspiration maneuvers[46,48], and more importantly for a longer period following a deep inspiration giving insights in the airway dynamics after deep inspirations. High resolution computed tomography (HRCT) scans visualizes the narrowing and distention of airways up to 3 mm following methacholine and deep inspirations, respectively[49]. These different techniques and study designs make it challenging to compare results between studies[50], but if these considerations are taken when reading study results on airway responses to deep inspiration a fair comparison can be made.

- Consider differences in airways obstruction (baseline vs. induced obstruction vs. spontaneous obstruction).
- Consider differences in method of measuring the response. FEV_1 includes a deep inspiration in the measurement and therefore may affect the response directly, but partial flow volume curves are less reproducible.

- Consider timing of the measurement following deep inspiration. In asthma it has been shown that airways are dilated by deep inspiration, but re-constrict within seconds. In addition, the bronchodilatory effect of deep inspiration holds for almost 1 minute in healthy subjects[46]. The outcome, therefore, depends greatly on the timing of the measurement following the deep inspiration.

Despite these differences many studies have been performed to examine the effects of deep inspiration on airway mechanics in healthy subjects and patients with asthma or other pulmonary diseases and have led to great insights. Deep inspirations not only have a dilatory effect on constricted airways, they also have a protective effect against airway narrowing.

2.4. Deep inspiration avoidance

In general, it has been shown that avoidance of deep inspirations for at least 20 minutes enhances subsequent airway narrowing to bronchoconstrictor stimuli in healthy subjects, whereas this is effect is not that profound in asthma[45]. However, prohibition of deep inspirations in healthy subjects makes the dose-response curves similar, but not equivalent to asthmatic subjects[51,52]. This suggests that deep inspirations have a protective effect against airway narrowing, and that this is lost in asthma[44,45,48,51,53,54].

The potentiating effect of deep inspiration avoidance on airway narrowing is subtrate-dependent. Whereas the reaction to methacholine is enhanced, the response to bradykinin is not altered by prohibition of deep inspirations in both healthy subjects and asthmatic patients[55]. The duration of deep breath avoidance also determines the subsequent bronchoconstrictor response[43], and the subsequent ability of deep inspirations to dilate the airways[44,45,48].

2.5. Deep inspiration-induced protection

Studies examining the protective effect of deep inspirations otherwise, showed that deep inspirations taken directly prior to inhalation of bronchoconstrictor stimuli prevented the subsequent airway narrowing in healthy subjects. This was not seen in patients with asthma or other disease groups with airway hyperresponsiveness[56]. The protective effect of deep inspiration is greater when more deep breaths are taken prior to spasmogen inhalation[43]. In addition, the duration of protection by deep breath seems to last longer than dilation induced by deep breath (6 vs. 1 min)[43,44,57].

In general, this led to the conclusion that deep inspirations protect against airway narrowing in healthy subjects, and that this protective effect is lost in asthma.

2.6. Deep inspiration-induced bronchodilation

Deep inspirations do not only protect against upcoming airway narrowing, they can also dilate constricted airways. This bronchodilatory effect is the effect of deep inspirations that is mostly studied[41,45,48,51,56,58,59]. When airway narrowing is induced in healthy subjects, either in absence

of deep inspirations or with high doses of bronchoconstrictor stimuli, deep inspirations can reduce the airway narrowing almost back to baseline levels[43,60].

This bronchodilatory effect is impaired in patients with asthma as well[56], and the impairment is associated with asthma severity[61], and spontaneous airways obstruction[62]. Mild asthmatics are able to dilate the airways by deep inspiration, but to a lesser extent than healthy control subjects, whereas the bronchodilatory effect is absent in severe asthma. During asthma exacerbations deep inspirations can even lead to bronchoconstriction. In other words, the response to deep inspiration in asthma is variable, ranging from bronchodilation to bronchoconstriction[56,58,63-65].

The extent of bronchodilation is directly related to the lung volume reached by the deep breath[34], and the number of deep inspirations[51]. Also the magnitude and stimulus of bronchoconstriction determines the response to deep inspiration[44,60]. For example, in asthma deep inspiration-induced bronchodilation is less during the late phase than during the early phase of the allergic asthmatic reaction[66].

Using measurements that can continuously track airway resistance during tidal breathing and deep inspiration it has been shown that renarrowing of airways following deep inspiration is faster in asthma than in healthy controls[46,48,67]. In addition, with high-resolution CT scans renarrowing of airways following deep breaths has been visualized in asthma, but was not seen in healthy subjects[59].

2.7. Deep inspiration-induced bronchoconstriction

Under baseline conditions (i.e. no induction of airways obstruction) deep inspirations can transiently induce bronchonstriction in patients with asthma[58,63,64]. This is not observed in healthy control subjects, or non-asthmatic allergic patients. During spontaneous asthma exacerbations deep inspirations result in profound bronchoconstriction related to the severity of the spontaneous obstruction[62]. Deep inspiration-induced bronchoconstriction resolved after treatment of the exacerbation with corticosteroids.

Thus, deep inspirations modulate airway responses to bronchoconstrictor agents and can therefore be considered as a very strong physiologic protective mechanism against airway narrowing. Indeed, the fact that the airways of asthmatic patients respond differently to lung inflation by deep inspiration indicates that the loss of this protective mechanism is involved in the pathophysiology of asthma. However, both the physiological mechanism responsible for lung volume-induced bronchoprotection in healthy subjects, as well as the pathophysiological mechanism of how this bronchoprotection is lost in asthma has not been elucidated yet. The next chapters resume the knowledge on these mechanisms thus far.

3. Physiological mechanism of deep inspirations

During lung inflation the diaphragm moves downwards and the thorax up-and outwards. The parenchyma follows these movements, and therefore the airways that are embedded in the parenchyma are dilated **(Figure 3)**[57,68-70].

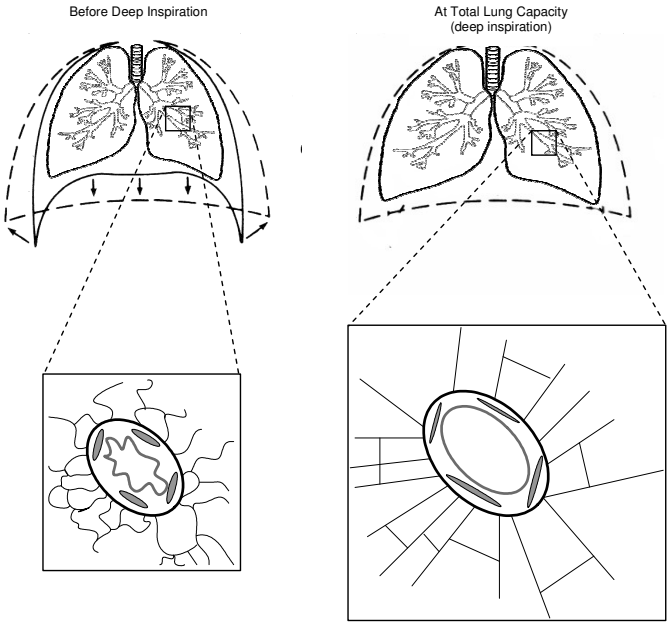


Figure 3. This figure shows that upon deep inspiration (upper figures) the airways embedded in the parenchyma are dilated as a result of parenchymal tethering (bottom figures).

Two forces potentially dilate the airways during lung inflation. One is the bulk modulus that is associated with the pressure difference acting directly across the airway wall, transmitted from the pleural space through the parenchyma, and relative to the intraluminal pressure for each airway. The other is the shear modulus, describing the force of local tethering, or pulling, by the surrounding lung parenchyma.

It is generally assumed that there is a tight coupling between lung volume changes and changes in airway caliber, as well as between airway caliber and smooth muscle length. Many of the processes described below follow this assumption.

3.1. Airway smooth muscle

Multiple investigators have linked the phenomena of deep breath-induced bronchodilation and bronchoprotection to the ability of deep inspirations to stretch the airway smooth muscle cell, thereby reducing its contractile capacities[36,45,48,71,72]. Notably, any explanation of *in vivo* phenomena, such as deep inspiration-induced bronchodilation and bronchoprotection, based

on evidence gathered from studies of isolated airway smooth muscle, can only be regarded as provisional until all interactions between ASM and other lung components are clear.

Airway smooth muscle and its contractile and cytoskeletal filaments are subjected to time-varying mechanical stress and strain associated with tidal lung inflations and spontaneous deep breaths. The forces serve to maintain airway caliber (distend or deform the cytoskeleton), but at the subcellular level also provide a mechanical stimulus to induce cellular responses, such as structural remodeling, and gene expression[73]. Consequently, the function of airway smooth muscle must be equilibrated with this dynamic environment[72].

The expected stretch of airway smooth muscle during tidal breathing is about 4%, and 12% during a sigh[72]. Stretch of airway smooth muscle of about 3% is enough to inhibit force generation by 50%, and therefore can be induced by the stretching forces of tidal breathing[72].

The effects of stretch on smooth muscle function, especially force generation, stiffness and shortening velocity, have been studied extensively. The effects are dependent on the speed[74,75], the amplitude, and the frequency[76] of the stretch imposed on the airway smooth muscle cell. However, the mechanism how stretch leads to less force generation is still under debate:

3.1.1. Bridge dynamics

The number of cross-bridges between actin and myosin determines the length change and force generation upon activation[77,78]. Stretch of the airways by lung inflation may lead to perturbation of these cross-bridges and thus in a reduction of contractility[71,72,79]. Although this theory explains deep inspiration-induced bronchodilation, it does not explain the bronchoprotective effect. This may be explained by the static and dynamic equilibrium of actin and myosin cross-bridge formation. When stretched adequately, the smooth muscle fibers are placed into disequilibrium, so that subsequent activation of smooth muscle is difficult[72].

3.1.2. Plasticity

Stretch induced changes in muscle force/tension may be caused by effects on contractile elements, as well as effects on the elastic elements in series with them. The muscle cell is able to adapt its contractile apparatus to length changes[36,80,81], which is called plasticity (rearranging the contractile properties to the new cell dimensions). This could explain bronchoprotection by deep inspirations, since the stretches would prevent adaptation to a certain length and therefore de-optimalize the length-force relationship. The structural basis and regulation mechanisms for such adaptation mechanism are still not known. A more integral approach to cell adaptation has been suggested by Fredberg[82], who considers the smooth muscle cell behavior like a soft glass. All the components of the smooth muscle cell are weakly interacting discrete elements. The ability to deform, flow and remodel is governed by nonthermal agitation (motion) energy of the cytoskeleton elements relative to the energies that constrain their motion[83,84]. A mechanical strain can act as an energy source that helps individual elements to

jump out of their energy well, so the cytoskeleton essentially 'heats up' and 'melts'. After each strain the system evolves into configurations that are more stiff and stable, and needs to be reversed by a subsequent stretch.

Although all these observations can result from direct physical effects of stretch imposed on smooth muscle cells it is also likely that stretch receptors coupled to chemical signaling pathways are involved.

3.2. Release of endogenous dilating substance (nitric oxide) from non-adrenergic, non-cholinergic nerves (Nitric Oxide, VIP)

Cholinergic and adrenergic systems control the bronchomotor tone together with the non-adrenergic non-cholinergic (NANC) system which mediates contraction (excitatoryNANC) or relaxation (inhibitoryNANC) of airway smooth muscle. Nitric oxide is the predominant neurotransmitter of the iNANC system[85]. In the respiratory tract, nitric oxide is produced by a wide variety of cell types and is generated via oxidation of L-arginine that is catalyzed by the enzyme nitric oxide synthase (NOS). Nitric oxide synthesis from endothelium is believed to be partly stretch-dependent[86], and thus may play a role in the deep inspiration dynamics since nitric oxide has bronchodilatory effects on methacholine induced bronchoconstriction[87-89]. Indeed, in anaesthetized and ventilated dogs a nitric oxide synthase inhibitor (N^G-nitro-L-arginine methyl ester) prevented bronchodilation by a large deep inspiration. These results suggest that a large inflation of the lung may normally releases of nitric oxide resulting in airway dilation[90]. Furthermore, a report only published in abstract format reported that in human subjects without asthma, inhalation of a nitric oxide synthase inhibitor (L-NAME) reduced the deep inspiration-induced bronchoprotection against methacholine-induced bronchoconstriction[91].

Whether the neuropeptide vasoactive intestinal peptide (VIP) plays a role in the beneficial effects of deep inspiration has not been investigated yet.

3.3. Neural activation

The parasympathetic nervous system mediates both cholinergic contractions and non-adrenergic, non-cholinergic (NANC) relaxations of airway smooth muscle. Activation of these pathways following chemical and/or mechanical stimulation of afferent nerves innervating the lungs can profoundly influence airway calibre and thus resistance to airflow[92]. Lung inflation induces neural activation. Increasing the volume or rate of lung inflation increases the discharge frequency of intrapulmonary stretch receptors[93]. This could explain the effects of rate and magnitude of stretch of the airways on airway tone. Namely, in healthy and asthmatic subjects fast inspirations reduce induced airway resistance more than slow inspirations[94]. Stretch receptor activation by deep inspiration may cause central inhibition of parasympathetic tone[92] leading to bronchodilation.

3.4. Release of surfactant

Surfactant reduces surface tension in the peripheral airways and help to maintain airway caliber[95]. Surfactant is produced by and secreted from alveolar type II cells, where it is stored in intracellular vesicles termed lamellar bodies. Direct distortion of the type II cell is a direct stimulus for secretion of surfactant *in vitro*[96], and thus stretch by lung inflation of the alveolar epithelium may trigger the release of surfactant. Indeed, in rats exercise (swimming) led to an increase in breathing rate and tidal volume resulting in a release of surfactant from distortion of alveolar type II cells, but also from another surfactant pool under sympathetic nerve control[97]. This suggests that lung volumes above tidal volume induces the release of surfactant by stretch and thus modulates airway caliber.

3.5. Release of autacoids

Autacoids are biological factors which act like local hormones, have a brief duration, act near the site of synthesis, and are not blood borne. Autacoids are primarily characterized by the effect they have upon smooth muscle. Prostaglandin E_2 (PGE_2) released in asthmatic airways has bronchodilator properties and inhibits allergen-induced bronchoconstriction and release of inflammatory mediators. Stretch of the airways by deep inspiration may lead to release of autacoids such as prostaglandins and atrial naturetic peptide (ANP). For example PGE_2 is released by lung inflation[98]. ANP is secreted by cardiac atria and lung tissue; it has a bronchodilator action in normal subjects[99] and patients with asthma[100], and has been shown to protect against histamine-induced bronchoconstriction in patients with asthma[101]. Also, intravenous infusion of ANP has been shown to cause bronchodilatation in patients with asthma[99], and inhaled ANP shows a strong dose-dependent protection against histamine-induced bronchoconstriction[102]. Of course, the question rises whether ANP is released upon stretch of the airways by lung inflation. Such evidence is not available, although circulating ANP levels rise upon exercise[101].

3.6. Hormonal pathway (adrenaline, epinephrine)

Circulating adrenaline is the only hormone known to influence bronchomotor tone. Adrenaline (or epinephrine) is released from the adrenal medulla, and induces bronchodilation by stimulating $beta_2$ adrenergic receptors on airway smooth muscle, and indirectly by reducing acetylcholine release[103]. Adrenaline is not released in response to allergen-induced or pharmacologically-induced bronchoconstriction. After exercise, increased adrenaline concentrations induce airway dilation. Infusion of epinephrine in normal non-atopic individuals comparable to exercise levels also results in bronchodilation[104,105]. which is probably a result of inhibition of vagal tone. There are no data on whether this is a direct result of increased volume changes during exercise or that increased adrenaline release is stretch-induced.

4. Pathophysiological mechanism

The effects of deep inspiration on airway caliber can become impaired by any factor that reduces the strain transmission from the parenchyma to the airway wall, leading to less airway dilation during lung inflation. On the other hand, even if the airways are stretched adequately during lung inflation, the airway wall, and its components, may respond differently to the stretch imposed on it (**Figure 4**).

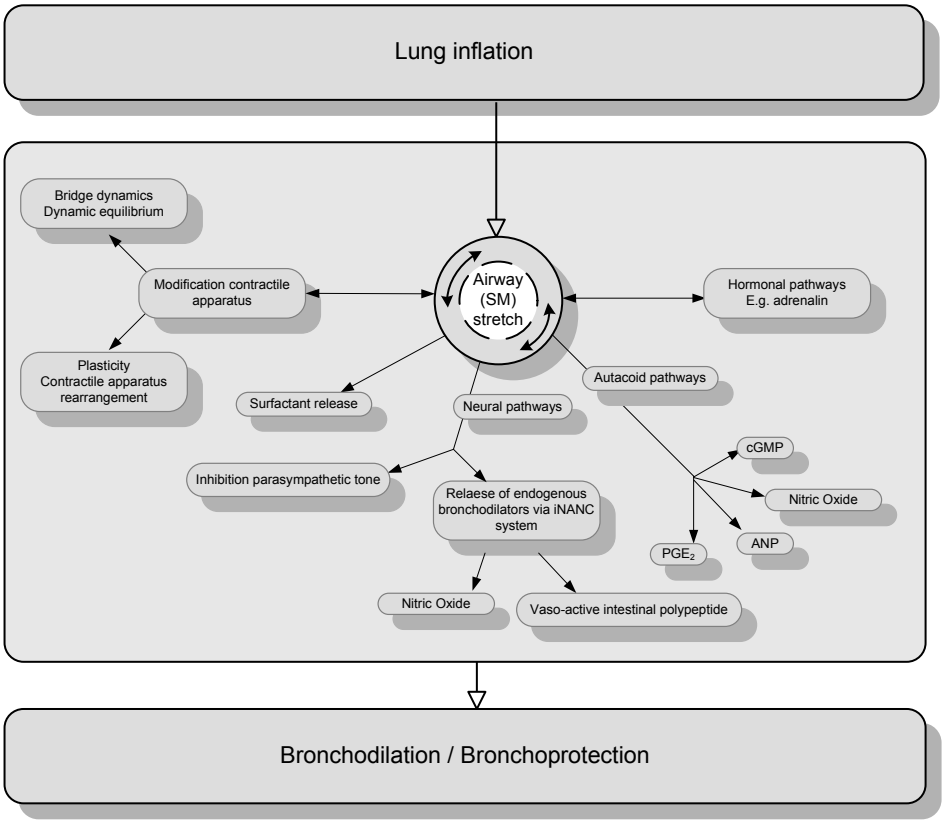


Figure 4. Possible mechanisms explaining beneficial effects of deep inspiration.
This figure shows the possible mechanisms explaining the beneficial effects of deep inspiration. First, lung inflation results in stretch of the airways and its components. This could lead to release of surfactant, activation of neural, autocoid or hormonal pathways, or modification of the contractile apparatus. On the other hand, changes in the contractile apparatus or hormonal pathways could also affect the amount of stretch induced by lung inflation. In the end, these proposed mechanisms determine the level of bronchodilation and/or bronchoprotection induced by lung inflation.

4.1. Impaired stretch

Stretch of the intrapulmonary airways, and thereby the smooth muscle, by lung inflation is caused mostly by the increase in radial traction exerted on the airways by the surrounding lung parenchyma[69,70,106]. The impairment of the beneficial effects of deep inspiration in asthma could therefore be caused by impaired coupling between the parenchyma and airways. Studies using high resolution CT (HRCT) are inconsistent with this hypothesis. These showed that the airways are stretched to the same extent in both healthy and mild asthmatic subjects. The groups were different with regard to the response following deep inspiration, resulting in bronchodilation and bronchoconstriction respectively[59]. However, in more severe asthmatic patients airway distensibility does seem to be impaired[48,61], and thus loss of airway-parenchyma interdependence may play a role in more severe asthma.

4.1.1. Increased stiffness of the airway wall

Change in airway distensibility may be due to remodeling in those proportions of the wall distinct to airway smooth muscle (ASM): adventitia, lamina propria, reticular layer under epithelium[3]. Thickening of the airway wall may have both beneficial[21,107-109] and detrimental[22,110] effects on airway mechanics during lung inflation, and airway hyperresponsiveness. Analysis of the effect of airway remodeling has to be based not only on geometry, but also on the mechanical properties of the altered airway wall components[28]. For example if deposited connective tissue in the adventitial layer is stiff, then it would attenuate the cyclical strain from the surrounding parenchyma. But if deposited matrix is highly compliant it would also prevent effective transmission of strain to the airway smooth muscle layer. Therefore, there must be an optimal coupling stiffness that allows maximal transmission of strain from the parenchyma to smooth muscle in order for the airways to receive maximal benefit of the bronchodilating effect of tidal breathing and deep inspirations. In asthma, airway wall remodeling occurs under inflammatory processes. Since it is impossible *in vivo* to examine the influence of the individual components of the airway wall on airway distensibility and the responses to stretch, airway models are needed to clearify the role of remodeling processes on airway mechanics during deep inspiration. So far, this role has not been fully elucidated.

4.1.2. Airway wall edema

Edema of the airway wall or within the peribronchial space could uncouple the interdependence between the airways and the parenchyma resulting in decreased radial forces acting on the smooth muscle during deep inspiration[55,111-113]. If so airway stretch by lung inflation would be limited. Substantial airway wall edema (up to 50% increase in airway wall area) by bradykinin or intravenous saline can be elicited in the airways. However, this potential effect of airway wall edema to decrease airway wall distention by lung inflation was not found in either dogs[114], or sheep[113,115]. On the other hand, in asthma increased transpulmonary pressure led to less bronchodilation by deep inspiration, whereas this effect was not seen in healthy subjects[116].

This may be due to fluid flux across leaky capillaries in inflamed asthmatic airways (thus not seen in healthy subjects) leading to direct loss of airway-parenchymal interdependence[116].

4.1.3. (Peri)Bronchial inflammation

(Peri)Bronchial inflammation would interfere with the distending forces of the parenchyma on the airway wall[117]. In addition it would also reduce the recoil pressures of the parenchyma, reducing the load on airway smooth muscle during contraction. Up till now no clear relationship between impairment of deep inspiration induced bronchodilation and inflammatory markers has been established. An inverse correlation between broncho-alveolar lavage (BAL) concentrations of eosinophils and deep inspiration-induced bronchodilation has been shown[118]. However, sputum inflammatory cell counts were not related to deep inspiration-induced bronchodilation[51]. Still several studies have shown that anti-inflammatory treatment improves deep-inspiration induced bronchodilation[119-121]. This could be due not only to reduction of the ongoing inflammatory process, but also to stretch-induced alterations in function of the smooth muscle itself[122,123].

4.1.4. Loss of alveolar attachments

The attachments of the parenchyma to the airway wall determine whether the transpulmonary pressures are transmitted across the airway wall. Patients who died of asthma have damaged alveolar attachments[3] which may lead to irreversible uncoupling of the expansive forces and the airways. Also in COPD loss of alveolar attachments have been described and related to less deep inspiration-induced bronchodilation[124].

4.1.5. Reduced inspiratory capacity

Any chronic change in lung volumes leading to a lower inspiratory capacity could be expected to affect airway mechanics during deep inspiration. A reduction in the magnitude of distention from FRC to total lung capacity (TLC) would lead to lower tethering forces on the airway wall by the parenchyma. Examples are obesity[125,126], supine position during bed rest[127], and hyperinflation due to chronic airways obstruction (COPD)[128]. Also, it has been suggested that prohibition of deep inspirations leads to airway closure in healthy subjects and that deep inspirations reopen these lung regions (i.e. reduce heterogeneity)[129].

4.2. Airway smooth muscle

The presence[130] and the normal function[131] of airway smooth muscle in the lung are still poorly understood. In comparison to striated muscle, we know little about how smooth muscle contracts. Therefore, it is also difficult to say whether airway smooth muscle function is altered in asthma and whether the smooth muscle responds differently to stretch. However, many hypotheses on the pathophysiological mechanism of stretch-induced modulation of airway smooth muscle function have been proposed.

4.2.1. Latch bridges

Airway remodeling may lead to reduced "force fluctuation amplitude", and thus airway smooth muscle may be subjected to diminished tidal forces. As a result the cycling of cross-bridges is reduced, and therefore the number of attached actin-myosin bridges and stiffness of the muscle is increased. Every tidal stretch has less power to perturb these slow bridges until a new static equilibrium is formed where the muscle becomes so stiff that also deep breaths are no longer able to unfreeze the muscle (=latch state)[72]. A lower amplitude (less inflation or other reasons for not transmitting force to smooth muscle cells) leads to increase in cross-bridges and thus force generation and stiffness.

4.2.2. Plasiticity

Airway smooth muscle is able to adapt its contractile apparatus to different cell lengths[132,133]. Chronic shortening[134] of airway smooth muscle (> 3 days) shifts the passive and active length-force relationships enabling it to generate the same force at a shorter length. If tidal breaths and occasional deep breaths fail to change the length of the smooth muscle cells the cell is allowed enough time to adapt itself to that length and regain force generation. In addition, this was associated to increases in passive stiffness and thus further diminishing the effects of cyclic stretch.

Rearrangement of contractile elements from a series to a parallel configuration[80,132,135] may alter airway smooth muscle respons to stretch as well. In case of a parallel configuration, stretch would not perturb the contractile filaments and force generation remains intact.

Also, abnormally long actin filaments may increase the range of sliding of contractile filaments without diminishing the overlap between myosin and actin filaments, and thus render the muscle more resistant to the relaxing effect of oscillatory stress[122,136]. Longer actin filaments were also associated with greater resistance of stimulated muscle to relax upon oscillatory strain in a computer model[137].

4.2.3. Increased airway smooth muscle tone

Several studies have shown that under both cyclic strain and increased airway smooth muscle tone (*in vitro*) cultured cells demonstrate increased stiffness, as well as increased contractility and shortening velocity. In asthma increased airway smooth muscle tone may lead to harmful uniaxial cyclic stretch, instead of harmless biaxial cyclic stretch, leading to enhanced recovery from acute large stretches[73,138].

4.2.4. Increased shortening velocity of ASM

The airways of patients with asthma reconstrict faster following deep inspiration as compared to healthy control subjects[46,48,67,139]. This suggests that the airway smooth muscle cells exhibit increased velocity of shortening, and thus contract faster after being stretched. This is in line

with *in vitro* studies demonstrating greater velocity of contraction of asthmatic or sensitized smooth muscle cells[140,141].

4.3. Alterations in neurohumoral system

4.3.1. Cholinergic system

In asthma deep inspirations may increase cholinergic tone and therefore lead to bronchoconstriction. Several studies support this hypothesis where pretreatment of asthmatic patients with anti-cholinergics attenuated subsequent deep-inspiration induced bronchoconstriction[63,142]. However, this effect can also be a result of the need for bronchotone to establish deep inspiration-induced bronchodilation. Indeed, in the presence of cholinergic blockade, but with reestablishment of bronchomotor tone with PGF2 alpha, deep inspiration-induced bronchodilation could still be demonstrated in healthy individuals[143].

4.3.2. iNANC/nitric oxide

NO synthase (NOS) catalyzes the oxidation of l-arginine to produce nitric oxide in the respiratory tract. NOS exists in three distinct isoforms: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS)[85,144]. NO derived from the constitutive isoforms of NOS (nNOS and eNOS) modulates airway tone. On the other hand, NO derived from iNOS seems to be a pro-inflammatory mediator with immunomodulatory effects. The concentration of this molecule in exhaled air is increased in asthma[145]. If nitric oxide is necessary for bronchodilation, and this is released by deep inspiration, then reduced availability of bronchodilatory nitric oxide under inflammatory circumstances and reduced stretch by deep inspiration may lead to less bronchodilation following deep inspiration.

The mechanism of how the beneficial effects of deep inspiration become impaired can be summarized in the same figure that shows how deep inspiration can lead to the beneficial effects (**Figure 5**). The pathways that are boxed have not yet been investigated *in vitro* or *in vivo* by intervention studies. The other pathways have been investigated but have not yet resulted in a definitive pathophysiological mechanism. Therefore major research questions and hypotheses remain to be examined regarding the (patho)physiological mechanism of deep inspiration dynamics.

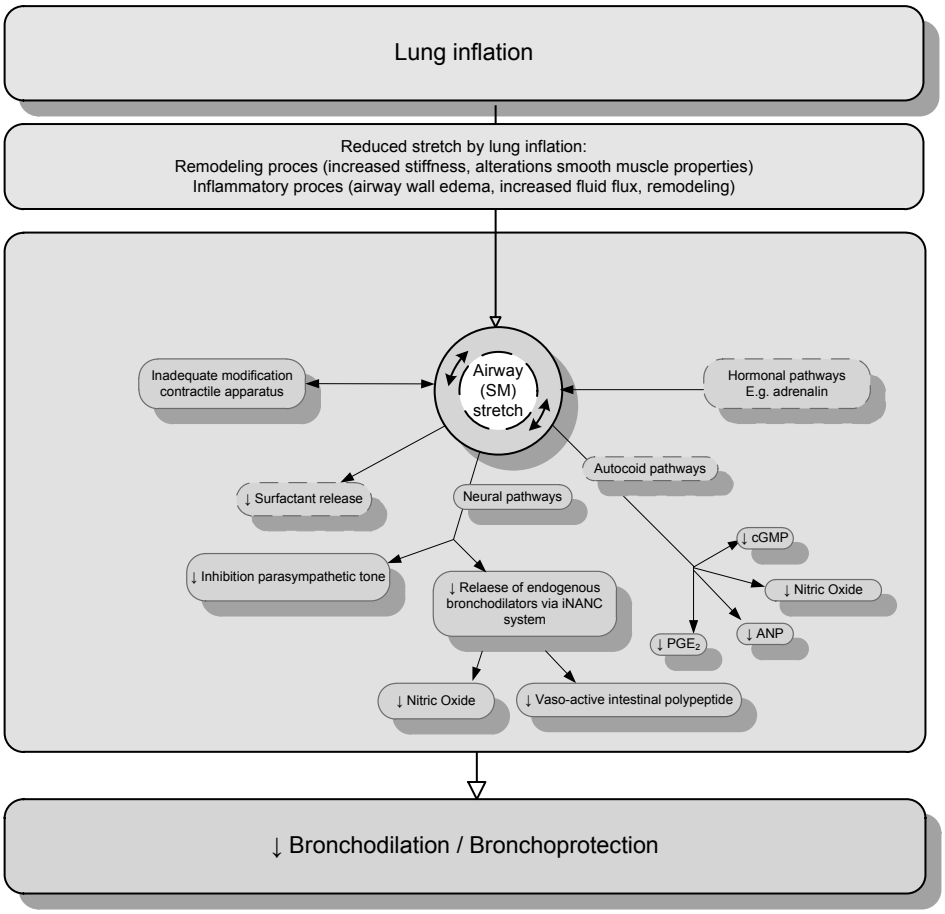


Figure 5. Possible mechanisms explaining impaired beneficial effects of deep inspiration.
This figure shows the possible mechanisms explaining impaired beneficial effects of deep inspiration. First, stretch of the airways and its components by lung inflation is reduced by the remodeling or inflammatory process within the airways. This could lead to reduced release of surfactant, changed activation of neural, atuocoid or hormonal pathways, or inadequate modification of the contractile apparatus. On the other hand, changes in the contractile apparatus or hormonal pathway could also affect the amount of stretch induced by lung inflation. In the end, these proposed mechanisms determine the level of bronchodilation and/or bronchoprotection induced by lung inflation. The pathways that are boxed have not yet been investigated *in vitro* or *in vivo* by intervention studies.

5. Rationale of the current thesis

In summary, deep inspirations provide the strongest endogenous protection against airway narrowing in healthy subjects, whereas this protective mechanism is lost in asthma. The physiological mechanism underlying the prevention and reduction of airways obstruction by deep inspirations in healthy subjects has not been elucidated. In addition, the pathophysiological

mechanism how this mechanism is impaired in asthma is unclear. In this thesis we addressed the following issues concerning the (patho)physiological mechanism of deep inspiration-induced bronchodilation and attempts to restore this mechanism.

Chronic airway inflammation in asthma can influence airway mechanics through the remodeling processes and thus impair deep inspiration-induced bronchodilation. The improvement of deep inspiration-induced bronchodilation by anti-inflammatory treatment in asthma is in line with this. As described above, this relationship has only been addressed by examining inflammatory cells in bronchoalveolar lavage fluid or sputum, but not in bronchial biopsies. In this thesis we aimed to further investigate the relationship between airway inflammation and airway responses to deep inspiration. We, therefore, investigated the relationship between the response of the airways to deep inspiration and airway inflammation measured in bronchial biopsies of patients with asthma. In addition, to examine whether impaired deep inspiration-induced bronchodilation is asthma-specific we compared these results with those from patients with chronic obstructive pulmonary disease (COPD) (chapter 2).

The airway smooth muscle cell has frequently been the center of the debate on deep inspiration-induced bronchodilation. It is thought that lung inflation stretches the smooth muscle cells within the airways and thereby changes the contractile apparatus of the cell, but the exact mechanism how the smooth muscle cell and/or the contractile apparatus is affected by stretch is unknown. Smooth muscle cells express several contractile and structural proteins. The level of expression and the type of proteins is depending on the functional phenotype of the cell, and is related to lung function in severe asthma. We examined the expression of several smooth muscle proteins that are likely to play a role in airway responsiveness in relation to deep inspiration-induced bronchodilation in asthma. In addition, we measured the expression of several components of the extracellular matrix, since the smooth muscle cell is likely to have a functional interaction with the surrounding extracellular matrix (chapter 3).

In asthma, during an exacerbation deep inspirations lead to further bronchoconstriction instead of relieving it. Edema of the airway wall has been suggested as one of the mechanisms leading to deep inspiration-induced bronchoconstriction. It is unclear whether airway wall edema *perse* leads to bronchoconstriction following deep breath or that the presence of airway inflammation, such as during an exacerbation, must be present as well Therefore, we aimed to study the airway responses to deep inspiration in patients with peribronchial edema due to mitral valve regurgitation in the absence of airway wall inflammation. In addition, we studied whether reduction of pulmonary congestion after mitral valve repair would alter airway responses to deep inspiration (chapter 4).

Restoring the physiological mechanism to prevent airway narrowing by lung inflation may provide more sustained asthma control, and reduce the need of current asthma treatment. We performed several intervention studies to investigate whether this protective mechanism could be restored in asthma. First, chronic ongoing inflammation and airway wall remodeling processes as seen in asthma can lead to reduced strain transmission from the parenchyma

to the airway wall or to an altered respons of the airway wall to the stretch imposed on it. Therefore, we aimed to maximally reduce airway inflammation in asthma by systemic corticosteroid therepy on top of inhaled corticosteroids in order to improve airway responses to deep inspiration by reducing airway wall thickness and therefore restoring the airway-parenchymal interdependence (chapter 5).

Also, anticholinergic drugs have been shown to protect against airway wall remodeling in animal models of allergic inflammation. It inhibited both airway smooth muscle proliferations, as well as smooth muscle contractility. Since the airway smooth muscle cell and its altered response to stretch has been frequently postulated as pathophysiological mechanism for impaired deep inspiration-induced bronchodilation, we hypothesized that treatment with a long-acting anticholinergic agent, tiotropium, in allergic asthma would improve bronchodilation by lung inflation (chapter 6).

Passive inflation instead of an active deep inspiration would induce stretch of the airways without large intrathoracic pressures and could therefore prevent extravasation of fluid in inflamed airways. In addition, the inflated volume may open closed airways, thereby redistributing tethering forces of the parenchyma on the airway wall leading to increased stretching forces. In the last intervention study we examined whether passive inflation could reverse airways obstruction in asthma, and thus restore this strong physiological protective mechanism against airway narrowing (chapter 7).

Finally, we have used time series of the respiratory system impedance data from the study shown in chapter 2 and 3 to study the respiratory system with high temporal resolution. Fluctuations in time series of respiratory system impedance measurements by forced oscillation technique exist in the healthy lung, and the variability of these fluctuations differs from an asthmatic lung. We hypothesized that the temporal course of respiratory system impedance is differentially affected by respiratory disease. In addition, we considered the impedance signal to arise from a dynamic system, and assumed that this system contains a deterministic component, that changes in distinct ways in respiratory diseases. In other words, a specific respiratory disease corresponds to changes in the control parameters that modify the dynamic behaviour of the system (chapter 8).

In the following chapters these studies are fully described and discussed separately. In the last chapter, the major results are summarized followed by a general discussion (chapter 9).

Study aims of this thesis

- In chapter 2, we compared the bronchodilatory and bronchoprotective effects of deep inspiration using the forced oscillation technique in patients with asthma, patients with COPD, and healthy control subjects. In addition, we investigated the relationship between the response of the airways to deep inspiration and bronchial wall inflammation in patients with asthma and COPD.
- We analyzed the bronchial biopsies from the study presented in chapter 2, and examined the relationship between airway responses to deep inspiration and the expression of structural and contractile markers of airway smooth muscle cells in patients with asthma, which is shown in chapter 3.
- In chapter 4, we examined the effect of airway wall edema on airway mechanics during deep inspiration in patients with mitral valve disease as compared to healthy subjects. In addition we explored whether this would change in absence of airway wall congestion following mitral valve repair.
- In chapter 5, we aimed to investigate the effects of high dose systemic corticosteroids on top of inhaled corticosteroids on deep inspiration-induced bronchodilation at a given level of airway narrowing in patients with asthma.
- In chapter 6, the results of anti-cholinergic treatment are shown on multiple parameters of lung function, including deep inspiration-induced bronchodilation and airway hyperresponsiveness.
- Chapter 7 presents the intervention study using passive inflation of the lungs to investigate whether this would improve bronchodilation as compared to an active deep inspiration in patients with asthma.
- Finally, in chapter 8 we evaluated fluctuations in time series of respiratory system impedance measurements by forced oscillation technique in the healthy and asthmatic lung to observe whether the temporal course of respiratory system impedance is differentially affected by respiratory disease, and whether the impedance signal arises from a dynamic system

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