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

Slats, A.M.

Chapter 9

Summary and general discussion

9.1. Introduction

Asthma is defined as a chronic inflammatory disorder, associated with airway hyperresponsiveness. As described in the general introduction deep inspirations modulate airway responses to bronchoconstrictor agents and can therefore be considered as a very strong endogenous protective mechanism against airway narrowing. The airways of asthmatic patients respond differently to lung inflation by deep inspiration resulting in less bronchodilation of constricted airways. The loss of this protective mechanism may be involved in the pathophysiology of asthma. The studies described in this thesis were all directed at either further elucidating the (patho)physiological mechanism underlying deep inspiration-mediated bronchoprotection, or to restoring this protective mechanism in asthma. A summary of the conclusions of the studies will be followed by a general discussion and directions for future research.

9.2. Summary

In chapter 2 and 3 the results are shown from an observational study to examine airway responses to deep inspiration in patients with asthma, COPD and healthy control subjects. We found that the bronchodilatory effect of deep inspiration is impaired in patients with asthma, and even more markedly impaired in patients with COPD, as compared with healthy subjects. It appears that the loss of deep inspiration-induced bronchodilation is not asthma specific. Whether it occurs from the same pathophysiological mechanism is unclear. However, in asthma, this impairment was related to the inflammatory cell numbers within the submucosa and airway smooth muscle layer. Namely, reduced deep inspiration-induced bronchodilation was associated with increased numbers of mast cells within the airway smooth muscle bundles and increased $CD4^+$ lymphocyte counts in the bronchial lamina propria (chapter 2). This association was not found in patients with COPD. In addition, in asthma impaired bronchodilation by deep inspirations was related to a lower level of expression of calponin, desmin, and MLCK expression in bronchial biopsies, whereas increased airway hyperresponsiveness was associated with a higher level of expression of α-SM-actin, desmin, and elastin in bronchial biopsies. Thus, airway hyperresponsiveness, lung function, and airway responses to deep inspiration are associated with the level of expression of some, but not all, of the smooth muscle contractile and structural proteins, as well as the composition of the extracellular matrix within the airway wall (chapter 3).

In chapter 4, we examined the effect of airway wall edema on airway responses to deep inspiration. This was done in patients with mitral valve regurgitation needing mitral valve repair surgery, since they are expected to have pulmonary congestion in the absence of allergic airway inflammation. We expected to observe an increase in respiratory resistance following a deep inspiration, as is seen in patients with asthma with spontaneous airways obstruction[1],

as a result of fluid flux across the airway wall as a result of large transpulmonary pressures during deep inspiration. However, in patients with mitral valve disease a deep inspiration did not lead to bronchoconstriction, although lung function was diminished as compared to healthy subjects. Thus, this suggests that airway wall edema *per se* may not lead to bronchoconstriction following deep inspiration (chapter 4).

Chapters 5, 6 and 7 show the results of studies addressing ways to restore the beneficial protective mechanism of deep inspirations against airway narrowing. First, we investigated whether maximal reduction of airway inflammation by a course of high-dose oral prednisone on top of inhaled corticosteroids in well controlled asthmatic patients would further improve deep inspiration-induced bronchodilation. Indeed, the degree of deep inspiration-induced bronchodilation at a given level of airways obstruction was improved by this treatment regimen. The improvement was not related to concurrent reductions in airway hyperresponsiveness or to changes in the level of exhaled NO (chapter 5). Second, anticholinergic drugs have been shown to protect against airway wall remodelling in animal models of allergic inflammation. It inhibited both airway smooth muscle proliferation, as well as smooth muscle contractility. We hypothesized that 21 days of treatment with tiotropium would improve lung function, airway hyperresponsiveness and deep inspiration-induced bronchodilation by inhibiting allergy-driven airway inflammation in asthma. Treatment with tiotropium did not significantly improve deep inspiration-induced bronchodilation or airway hyperresponsiveness, but a significant effect on baseline bronchial tone was observed. This was shown by improvements in both FEV_1/FVC ratio and FEV_1 % predicted (chapter 6). And finally, in chapter 7 we aimed to dilate constricted airways by using passive inflation with positive-pressure inflation in mild asthma. In addition, we examined whether this would restore bronchodilation by lung inflation in patients with asthma who showed no significant bronchodilation by an active deep inspiration. We showed that airways obstruction can indeed be reduced by positive-pressure inflation of the lungs in asthma, comparable to active deep inspiration. And this could also be achieved in patients with asthma who were not capable of significantly reducing airways obstruction by an active deep inspiration (chapter 7).

Finally, in chapter 8 we have used time series of the respiratory system impedance data from the studies 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[2]. 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 different respiratory disease. In other words, a specific respiratory disease corresponds to changes in the control parameters that modify the dynamic behavior of the system. Using cross-validated linear discriminant analysis on mean Zrs, Rrs and Xrs enabled us to classify COPD vs. healthy controls, and asthma vs. COPD.

The distance-based analysis shows further evidence that there are differences in respiratory properties between asthma and COPD. Differences in the shape of the dynamic behavior were sufficient to correctly classify 80% of subjects to be either asthmatic or COPD patient (cross-validated). These findings are in keeping with the hypothesis that the two diseases affect the within-breath dynamics of respiratory impedance in a different way (chapter 8).

Taken together, we may conclude that specific inflammatory cells within the airway submucosa and airway smooth muscle layer, as well as the level of expression of specific smooth muscle and extracellular proteins may alter the airway responses to deep inspirations, but

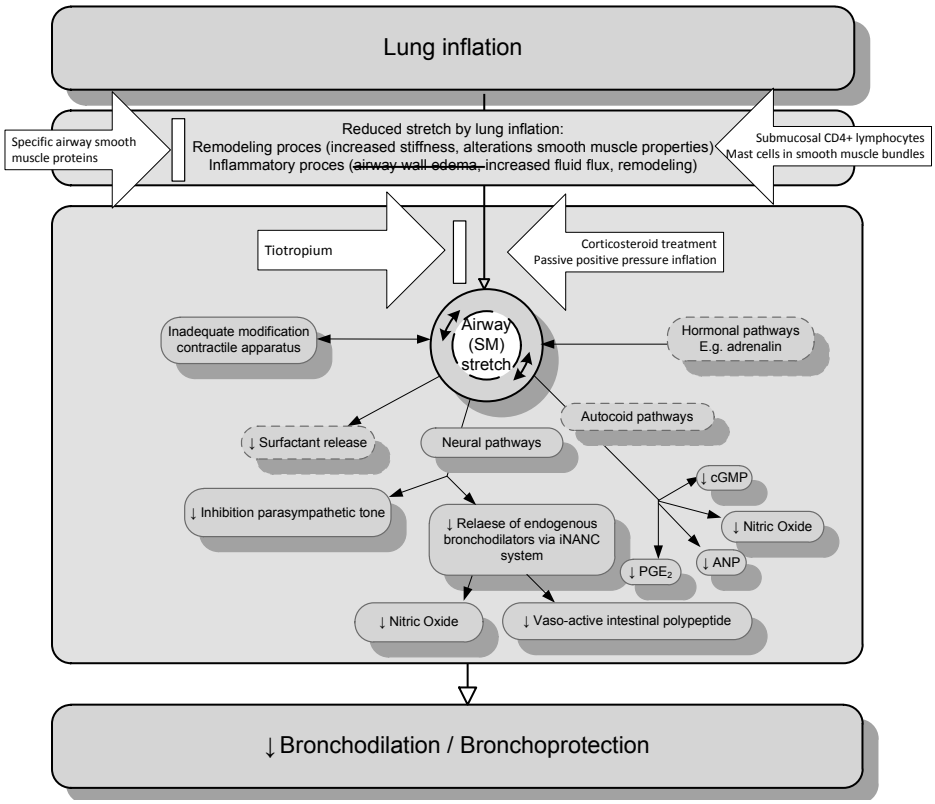

Figure 1. Possible mechanisms explaining impaired beneficial effects of deep inspiration.
This figure is derived from **figure 5 in chapter 1**. It shows the possible mechanisms explaining impaired beneficial effects of deep inspiration. In addition, the arrows show the main results of our studies and where they interact with these mechanisms. First, the level of expression of specific airway smooth muscle proteins, nor treatment with tiotropium-bromide, seems to interfere with the amount of stretch of the airways that is induced by lung inflation, and thus the degree of bronchodilation upon deep inspiration is not affected. On the other hand, the number of CD4+ lymphocytes in submucosa and mast cells in smooth muscle bundles may affect the remodeling and/or inflammatory process in the airway wall and thereby affect airway distensibility upon deep inspiration. Maximal steroid therapy and passive positive pressure inflation increased bronchodilation, most likely through increasing airway stretch upon deep inspiration.

edema *per se* does not. In addition, steroids and passive positive pressure inflation improve bronchodilation of constricted airways by deep inspiration, whereas tiotropium does not exert this effect (**Figure 1**). And finally, studying impedance data with high temporal resolution shows that the dynamic behavior of the respiratory system differs between asthma and COPD. These findings will be further discussed in relation with other published data in the following sections.

9.3. Deep inspiration-induced bronchodilation, airway inflammation and steroid responses

In chapter 2 we found that in asthma increased numbers of CD4 + lymphocytes within the submucosa, and mast cells within the airway smooth muscle bundles, are related to less bronchodilation following a deep breath. Furthermore, in chapter 5 we showed that high dose prednisolone on top of inhaled corticosteroids significantly improved deep inspiration-induced bronchodilation of constricted airways. Are these phenomena linked? In other words is the improvement by steroid therapy likely a result of inhibition of submucosal CD4+ lymphocytes or mast cells in the smooth muscle layer?

9.3.1. CD4+ lymphocytes

First, steroids have been shown to reduce CD4+ lymphocytes. For example, in allergic asthmatic patients pre-treatment with prednisone (3 days prednisone 30mg twice daily) inhibited influx of inflammatory cells, specifically eosinophils, basophils and CD4+ lymphocytes, in broncho-alveolar lavage fluid after segmental allergen challenge[3]. Another placebo-controlled study showed that a longer treatment protocol with oral corticosteroids (prednisolone 20mg o.d. for 2 weeks followed by 10mg for 4 weeks) significantly reduced asthma symptoms, albuterol usage, and increased FEV1 in steroid naïve asthmatic patients. This was accompanied by a reduction in submucosal eosinophils (81%), mast cells (62%) and CD4+ lymphocytes (68%), whereas placebo treatment resulted in no significant changes in cell numbers in the bronchial biopsies. The reduction in CD4+ lymphocytes was related to a decrease in airway hyperresponsiveness[4]. Also, inhaled corticosteroids suppress airway inflammation by ongoing allergen challenge with low-dose house dust mite in mild asthmatic patients, especially eosinophils, neutrophils, and lymphocytes[5].

On the other hand, in patients with asthma withdrawal of inhaled corticosteroid therapy (1760 mcg/day), until peak flow dropped by 25%, FEV1 dropped by 15% or 6 weeks elapsed, showed that half of the patients exacerbated and half of them did not. In both groups eosinophils increased in bronchial biopsies by steroid withdrawal, but CD4+ lymphocytes increased only in the groups that exacerbated[6]. This suggests that the steroid withdrawal-induced increase of CD4+ lymphocytes exert a greater influence on the airway function than eosinophils, and may

explain why we did not find a relationship between eosinophils and airway responses to deep inspiration. Taken together, it is plausible that steroid-induced improvement in bronchodilation by deep inspiration is mediated through inhibition of submucosal CD4 lymphocyte infiltration.

9.3.2. Mast cells in airway smooth muscle bundles

What about mast cells in airway smooth muscle bundles? In asthma, as compared to non-asthmatic subjects, the airway smooth muscle bundles are infiltrated by increased numbers of mast cells and lymphocytes[7]. Mast cells are recruited to the airway smooth muscle bundles by numerous chemo attractants (stem cell factor, chemokines, cytokines, CCR3 and CxCR1)[8]. In contrast, non-asthmatic airway smooth muscle releases mediators that inhibit mast cell migration towards asthmatic airway smooth muscle[8]. The presence of mast cells within the airway smooth muscle layer has been associated with airway hyperresponsiveness in asthma, but not in subjects with eosinophilic bronchitis[9,10]. Furthermore, dexamethasone-treated smooth muscle cells were less effective in enhancing C3a-induced mast cell degranulation and thus may lead to less bronchoconstriction[11]. But whether mast cell migration to airway smooth muscle is reduced by steroid treatment has not been investigated yet. Therefore, no direct conclusions can be made whether steroid-treatment in asthma improves deep inspiration-induced bronchodilation by reducing mast cell infiltration or degranulation in airway smooth muscle bundles.

9.3.3. Steroids and airway smooth muscle cell relengthening

Although the beneficial effects of corticosteroids have been attributed to suppression of airway inflammation it is possible that steroid-treatment exerts direct action on airway smooth muscle cells as well and thereby improves airway hyperresponsiveness and deep inspiration-induced bronchodilation. It has been shown that force fluctuations imposed on contracted airway smooth muscle cells in vitro results in relengthening of the cells[12], and is regulated through the p38MAPkinase signaling pathway[13]. Corticosteroids inhibit p38MAPkinase signaling and indeed augment force fluctuation-induced relengthening of airway smooth muscle cells *in vitro*[14]. Steroid-treatment could therefore have improved deep inspiration-induced bronchodilation by augmenting relengthening of airway smooth muscle cells upon stretch by inhibiting p38MAPkinase. However, this is still speculative since we did not measure p38MAPkinase in our studies.

9.4. Deep inspiration-induced bronchodilation and airway smooth muscle cells

As shown in the introduction, there are many hypotheses on the role of airway smooth muscle in the (patho)physiological of deep inspiration-induced bronchodilation (chapter 1; 3.1 and

4.2). By examining the relationship between airway responses to deep inspiration and the expression of structural and contractile markers of airway smooth muscle cells in bronchial biopsies of patients with asthma as shown in chapter 3 we aimed to further elucidate this role. We found that more bronchodilation by deep inspirations was related to higher levels of expression of calponin, desmin, and MLCK in bronchial biopsies, whereas increased airway hyperresponsiveness was associated with a higher level of expression of α-SM-actin, desmin, and elastin. Are our results in support of the previously presented hypotheses?

9.4.1. Plasticity

Plasticity refers to the ability of airway smooth muscle cell to adapt its contractile apparatus to the length at which it is activated. Smooth muscle cells with longer actin filaments show a more elastic behavior by increasing the range of myosin-actin overlap, which can generate the same amount of force after being stretched[15] and may therefore increase airway hyperresponsiveness. A higher level of α-SM-actin expression in bronchial biopsies may indicate more actin monomers that can form longer actin filaments by polymerization in the asthmatic inflammatory environment, and thus supports the relationship we found with airway hyperresponsiveness. In contrast, we would have expected to find increased levels of α-SM-actin expression in patients with less bronchodilation after a deep breath, but found the opposite. Increased α-SM-actin expression, in combination with increased levels of desmin and elastin expression, may therefore determine force generation upon stimulation but not stretch-induced bronchodilation. It is possible that more factors, such as extra-cellular matrix composition, airway wall thickness, or number of alveolar attachments determine the net bronchodilatory effect of lung inflation.

9.4.2. Increased smooth muscle tone

Interestingly, we found a positive relationship between FEV_1% predicted and the positive staining intensity for the smooth muscle proteins calponin, desmin, and MLCK, as well as a negative relationship between these markers and deep breath–induced reduction in respiratory resistance. It has been shown that cultured smooth muscle cells with increased tone produce enhanced levels of contractile proteins, such as myosin, MLCK and desmin, when cultured under cyclic stretch conditions[16]. The positive correlations between FEV_1% predicted and deep breath–induced bronchodilation could therefore reflect the effect of stretch on contractile protein production in these patients with asthma, rather than the influence of increased expression of these contractile markers on airway function. Most *in vitro* studies on the effect of length changes have been performed in isolated tracheal smooth muscle strips and provide length oscillations[17,18]. A recent study in an isolated intact bronchial airway with luminal volume oscillations showed no bronchodilation by increasing oscillatory strains. It demonstrates the difficulty of testing airway wall contraction and dilation at airway smooth muscle cellular level, since the lung has such a unique geometry and structure[19]. Therefore, human *in vivo*

experiments, such as the recent studies, are most likely to reflect the "true" pathophysiology of the airways in asthma.

9.5. Intervention studies

Although research on (patho)physiological mechanisms is both interesting and necessary, in clinical research the inevitable question is "what is the clinical relevance?'. Since deep inspirations have shown to provide a physiological protective mechanism against airway narrowing (chapter 1) and that this beneficial effect is lost in asthma this clinical relevance is very close. Namely, if this mechanism can be restored in asthma it could provide the best combat to bronchospasms, and thus symptoms, and possibly reduce the need of current medical treatment. Unfortunately, restoring deep inspiration-induced bronchodilation and/or bronchoprotection in asthma of which the (patho)physiological mechanism is not completely understood may not be possible yet. On the other hand, trying to restore this mechanism could also lead to new insights on underlying pathophysiology.

Several studies in this thesis have intervened with parts of the pathophysiological mechanism in order to at least improve deep inspiration-induced bronchodilation. We studied the effect of maximal steroid treatment (chapter 5), anticholinergic treatment (chapter 6), and passive inflation (chapter 7) on airway responses to deep inspiration. Each of these studies interacted with possible causes of impaired stretch of the airways by lung inflation **(Figure 1)**. Maximal steroid treatment could reduce airway wall remodeling as well as inflammatory induced airway wall thickening. Anticholinergic treatment has shown to reduce allergy driven airway smooth muscle proliferation and contractility. And, passive inflation may stretch the airways more effectively plus to a larger extent by 'pushing from the inside' with positive pressure inflation than what can be achieved by 'pulling from the outside' with negative intra-thoracic pressure. In addition, anticholinergic treatment and passive inflation could also have changed the effect of stretch on the contractile apparatus of the smooth muscle cell, although we have not directly measured that. Below, these interventions are discussed, as well as bronchial thermoplasty, a novel option in asthma treatment.

9.5.1. Maximal steroid treatment

The improvement in deep inspiration-induced bronchodilation, as measured by improvement in M/P ratio, in asthma patients by the use of systemic steroid treatment on top of maintenance therapy with inhaled steroids was a novel finding. Previously, several studies already showed that inhaled corticosteroid in steroid-naïve asthma patients improved bronchodilation following deep inspiration[20,21], whereas another study found no effect of inhaled corticosteroids on the bronchodilatory effect of a deep inspiration in a study with asthmatic patients with mild-to-severe airway hyperresponsiveness. The methods of assessing the airway responses to deep

inspiration differed among these three studies, as well as the dose and type of inhaled steroids used. This emphasizes the need for more standardized measurements of deep inspiration-induced bronchodilation, preferably measurements not including a deep breath such as the forced oscillation technique. Furthermore, the results implicate that patients with asthma, who are regularly treated with inhaled corticosteroids, can have residual airway inflammation[22,23] that impairs the mechanical properties of the airways even during clinically stable episodes. Patients with impaired airway responses to deep inspiration, although being clinically stable, may therefore be more at risk for the development of exacerbations. As airway responses to deep inspiration tend to be related to asthma severity[24] and the severity of breathlessness[25], long-term studies are required in order to address the prognostic implications of impaired responses of the airways to deep inspiration in asthma patients.

9.5.2. Anticholinergic treatment

Recent meta-analysis indicating that usage of long-acting beta-agonists without concomitant inhaled corticosteroids increases the risk of asthma-mortality[26,27] has given way for developing studies on alternative bronchodilatory treatment in asthma, such as tiotropium. Interestingly, anticholinergics have also been shown to inhibit airway smooth muscle proliferation and contractility in models of allergic inflammation[28,29]. Therefore, we hypothesized that in allergic asthma daily use of tiotropium reduces smooth muscle contractility, thereby improving lung function, airway hyperresponsiveness and deep inspiration-induced bronchodilation. Treatment with tiotropium did not significantly improve deep inspiration-induced bronchodilation or airway hyperresponsiveness, but a significant effect on baseline bronchial tone was observed. Improvements in lung function have been shown by tiotropium added to conventional therapy in severe asthma. Long-term effects of anticholinergic treatment on airway hyperresponsiveness to a non-cholinergic agent has not been investigated before, although acute protection against histamine-induced bronchoconstriction has been shown by pre-treatment with ipatropium-bromide in normal subjects[30], possibly via inhibition actelycholine after vagal reflex release by histamine[31]. An inhibitory effect of tiotropium on airway smooth muscle cells has been found in ovalbumin challenged guinea pigs and not in non-challenged animals[28]. This might be due to an increased level of acetylcholine by an augmented acetylcholine release after allergen challenge, reduction of inhibitory M2 receptors, or nonneuronal release of acetylcholine in conditions of allergic inflammation[32]. In the absence of inflammatory mediators or growth factors increased levels of acetylcholine may not result in structural changes within the airways and thus no inhibitory effect of tiotropium can be observed. Therefore it is possible that in these mild asthmatic patients a lower level of inflammatory mediators was present within the airways and thereby reducing the inhibitory effect of daily tiotropium inhalation. Since we did not include induced sputum or bronchial biopsies as outcomes of this study we cannot relate this to the improvement in lung function we found in our study. Taken together, tiotropium has bronchodilatory effects both acute and prolonged in mild asthmatic patients

and may therefore be considered as alternative long-acting bronchodilator. Whether the observed changes in lung function and airway hyperresponsiveness are a result of sustained bronchodilation by muscarinic receptor blockage or inhibition of allergen-induced remodeling remains to be established.

9.5.3. Improving stretch by positive pressure lung inflation

We postulated that airway wall distension can be improved by manipulation of the intrathoracic pressures by passive lung inflation in patients with asthma. Mechanical inflation of the lungs would induce stretch of the airways without large subatmospheric 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. Indeed, we showed that positive pressure inflation resulted in more bronchodilation, as compared to an active deep inspiration in patients with asthma. The opposite has been done as well by adding a resistance to a deep inspiration, which resulted in lower airway conductance in patients with asthma as compared to a regular deep inspiration, but not in healthy subjects[33]. Since we did not measure transpulmonary pressures it is difficult to discriminate whether the bronchodilatory effect of positive pressure inflation was a result of actual dilation of the airway tree or reopening of closed airways that could not be opened by active deep inspiration. The relationship between the increase in inspiratory volume and improvement in bronchodilation following positive pressure inflation, suggests that a lower inspiratory volume by any cause could result in reduced bronchodilation following lung inflation. Instead of improving lung inflation-induced stretch of the airways, the reverse has been investigated as well. For example by strapping the chest wall, which reduced lung function[34], increased airway constriction to methacholine[35], and indeed reduced deep inspiration-induced bronchodilation[36]. Also, several studies on airway responses to deep inspiration have been performed in obese patients, who are known to have reduced inspiratory capacity. Obese asthmatics, but not lean asthmatics or non-asthmatic obese subjects, show an increase in airway resistance following a deep breath[37]. Also, obese non-asthmatic subjects show no bronchoprotective effects of deep inspiration as compared to non-obese non-asthmatic subjects, indicating that obesity alone alters airway mechanics during deep inspirations[38], possibly as a result of lower inspiratory volume capacities. Our data suggest that the tethering forces of the parenchyma during active deep inspiration, possibly in relation to the magnitude of the inspired volume, are not strong enough to adequately stretch the airway wall, which may be overcome by positive-pressure inflation, and could be applicated to non-asthmatic disorders resulting in lower inspiratory volumes as well, such as obesity.

9.5.4. Bronchial thermoplasty

Airway smooth muscle has been mentioned to be the appendix of the lung, in other words it may be useless and possibly harmfull[39]. A new intervention called bronchial thermoplasty

delivers controlled thermal energy to the airway wall resulting in prolonged reduction/loss of airway smooth muscle mass[40]. There is some evidence that it improves overall asthma control, as shown by improvement in quality of life, asthma symptoms, severe exacerbations, and health care utilization[41-44]. In addition, initially it appeared that airway hyperresponsiveness was reduced by bronchial thermoplasty as well[45], but placebo controlled studies could not demonstrate this. In dogs, bronchial thermoplasty led to increased airway size in both relaxed and contracted states over a normal range of inflation pressures[46], and reduced airway closure to methacholine[47] as shown with high-resolution CT scans. Whether airway responses to deep inspiration are affected by bronchial thermoplasty remains to be investigated. However, it can be postulated that if indeed airway smooth muscle content is decreased in the treated airways, that in pharmacologically constricted airways the contraction can more easily be overcome by lung inflation, and might even be comparable to the response of airways to deep inspiration in healthy controls.

9.6. Dynamic behavior of the respiratory system

Another approach to lung disease is by system biology[48,49]. The lung is a complex organ, and system biology integrates information from all levels of structure and function of the system[50-52]. A characteristic of biological systems is their temporal behavior. Studying the temporal behavior of the lung by analysis of fluctuations in airway function over time is a way to approach lung disease as a biological system[53]. Variability analysis of the respiratory system has been successfully applied on impedance data[2] and peak flow measurements before[54]. We used time series of impedance measurements by forced oscillation technique to examine this temporal behavior in asthma and whether this method allowed us to discriminate between asthma and COPD. The non-linear distance-based time series analysis of respiratory impedance led to a correct classification of patients with asthma or COPD in at least 80% of cases. This suggests that the within-breath dynamics of respiratory impedance as captured by forced oscillation technique provides alternative information on characterizing patients with obstructive lung disease. In addition, this method could be used to monitor airway disease and evaluate treatment.

9.7. Future research

The studies described in this thesis have helped to provide more insight into the (patho)physiological mechanism of deep inspiration-induced bronchodilation and have shown that standard therapy (glucocorticosteroids), and novel therapy (passive positive pressure inflation) improves the airway responses to deep inspiration. However, many questions remain before fully understanding the bronchoprotective effects of deep inspirations. First of all, the contractile

apparatus of the airway smooth muscle and its function over different lengths needs to be clarified in order to understand how deep inspiration-induced stretch can influence it. Second, the interaction of inflammatory cells with smooth muscle cells and the functional consequences should be addressed. And finally, direct interventional studies intended to improve airway function and airway responses to deep inspiration should be extended. These considerations and the results of the studies in this thesis led to these research questions:

- Are mast cells within the smooth muscle layer influenced by standard asthma therapy, and is this related to improvements in airway function and deep inspiration dynamics?
- Is it useful to create new therapies targeting specific airway smooth muscle proteins, such as actin or desmin to reduce airway hyperresponsiveness?
- Is extra-cellular matrix deposition in airway smooth muscle bundles responsible for impaired airway responses to deep inspiration?
- Do steroids also have an acute effect on deep inspiration-induced bronchodilation *in vivo* by stretch-induced relengthening of airway smooth muscle cells as has been shown *in vitro*?
- Could passive positive pressure inflation be useful in clinical practice, such as in the emergency unit for acute asthma exacerbations?
- Would passive positive pressure inflation improve airway responses to deep inspiration in other pulmonary diseases or disorders associated with lower inspiratory volume capacity as well?
- Does tiotropium indeed inhibit allergy driven airway smooth muscle proliferation *in vivo* in asthma?
- Do changes in the temporal behavior of airway function provide information on the course of the disease and treatment effects?

9.7. Final remarks

Deep inspirations provide a strong physiological endogenous protection against airway narrowing, which is lost in asthma, and other pulmonary diseases such as COPD. This thesis has provided further insight in the (patho)physiological mechanism underlying the beneficial effects of deep inspirations, as well as treatment options to restore this mechanism.

References

1. Lim, T. K., S. M. Ang, T. H. Rossing, E. P. Ingenito, and R. H. Ingram, Jr. The effects of deep inhalation on maximal expiratory flow during intensive treatment of spontaneous asthmatic episodes. *Am.Rev. Respir.Dis.* 1989; 140: 340-343.
2. Que, C. L., C. M. Kenyon, R. Olivenstein, P. T. Macklem, and G. N. Maksym. Homeokinesis and short-term variability of human airway caliber. *J.Appl.Physiol* 2001; 91: 1131-1141.
3. Liu, M. C., D. Proud, L. M. Lichtenstein, W. C. Hubbard, B. S. Bochner, B. A. Stealey, L. Breslin, H. Xiao, L. R. Freidhoff, J. T. Schroeder, and R. P. Schleimer. Effects of prednisone on the cellular responses and release of cytokines and mediators after segmental allergen challenge of asthmatic subjects. *J.Allergy Clin.Immunol.* 2001; 108: 29-38.
4. Djukanovic, R., S. Homeyard, C. Gratziou, J. Madden, A. Walls, S. Montefort, D. Peroni, R. Polosa, S. Holgate, and P. Howarth. The effect of treatment with oral corticosteroids on asthma symptoms and airway inflammation. *Am.J.Respir.Crit Care Med.* 1997; 155: 826-832.
5. J. de Kluyver, J. A. Schrumpf, C. E. Evertse, J. K. Sont, P. J. Roughley, K. F. Rabe, P. S. Hiemstra, T. Mauad, and P. J. Sterk. Bronchial matrix and inflammation respond to inhaled steroids despite ongoing allergen exposure in asthma. *Clin.Exp.Allergy* 2005; 35: 1361-1369.
6. Castro, M., S. R. Bloch, M. V. Jenkerson, S. DeMartino, D. L. Hamilos, R. B. Cochran, X. E. Zhang, H. Wang, J. P. Bradley, K. B. Schechtman, and M. J. Holtzman. Asthma exacerbations after glucocorticoid withdrawal reflects T cell recruitment to the airway. *Am.J.Respir.Crit Care Med.* 2004; 169: 842-849.
7. Begueret, H., P. Berger, J. M. Vernejoux, L. Dubuisson, R. Marthan, and J. M. Tunon-de-Lara. Inflammation of bronchial smooth muscle in allergic asthma. *Thorax* 2007; 62: 8-15.
8. Sutcliffe, A., D. Kaur, S. Page, L. Woodman, C. L. Armour, M. Baraket, P. Bradding, J. M. Hughes, and C. E. Brightling. Mast cell migration to Th2 stimulated airway smooth muscle from asthmatics. *Thorax* 2006; 61: 657-662.
9. Brightling, C. E., P. Bradding, F. A. Symon, S. T. Holgate, A. J. Wardlaw, and I. D. Pavord. Mast-cell infiltration of airway smooth muscle in asthma. *N.Engl.J.Med.* 2002; 346: 1699-1705.
10. Siddiqui, S., V. Mistry, C. Doe, K. Roach, A. Morgan, A. Wardlaw, I. Pavord, P. Bradding, and C. Brightling. Airway hyperresponsiveness is dissociated from airway wall structural remodeling. *J.Allergy Clin. Immunol.* 2008; 122: 335-341.
11. Thangam, E. B., R. T. Venkatesha, A. K. Zaidi, K. L. Jordan-Sciutto, D. A. Goncharov, V. P. Krymskaya, Y. Amrani, R. A. Panettieri, Jr., and H. Ali. Airway smooth muscle cells enhance C3a-induced mast cell degranulation following cell-cell contact. *FASEB J.* 2005; 19: 798-800.
12. Mitchell, R. W., M. L. Dowell, J. Solway, and O. J. Lakser. Force fluctuation-induced relengthening of acetylcholine-contracted airway smooth muscle. *Proc.Am.Thorac.Soc.* 2008; 5: 68-72.
13. Lakser, O. J., R. P. Lindeman, and J. J. Fredberg. Inhibition of the p38 MAP kinase pathway destabilizes smooth muscle length during physiological loading. *Am.J.Physiol Lung Cell Mol.Physiol* 2002; 282: L1117-L1121.
14. Lakser, O. J., M. L. Dowell, F. L. Hoyte, B. Chen, T. L. Lavoie, C. Ferreira, L. H. Pinto, N. O. Dulin, P. Kogut, J. Churchill, R. W. Mitchell, and J. Solway. Steroids augment relengthening of contracted airway smooth muscle: potential additional mechanism of benefit in asthma. *Eur.Respir.J.* 2008; 32: 1224-1230.
15. Wang, L., P. Chitano, and T. M. Murphy. Length oscillation induces force potentiation in infant guinea pig airway smooth muscle. *Am.J.Physiol Lung Cell Mol.Physiol* 2005; 289: L909-L915.
16. Maksym, G. N., L. Deng, N. J. Fairbank, C. A. Lall, and S. C. Connolly. Beneficial and harmful effects of oscillatory mechanical strain on airway smooth muscle. *Can.J.Physiol Pharmacol.* 2005; 83: 913-922.
17. Fredberg, J. J., D. Inouye, B. Miller, M. Nathan, S. Jafari, S. H. Raboudi, J. P. Butler, and S. A. Shore. Airway smooth muscle, tidal stretches, and dynamically determined contractile states. *Am.J.Respir.Crit Care Med.* 1997; 156: 1752-1759.
18. Wang, L., P. D. Pare, and C. Y. Seow. Effects of length oscillation on the subsequent force development in swine tracheal smooth muscle. *J.Appl.Physiol* 2000; 88: 2246-2250.

19. LaPrad, A. S., T. L. Szabo, B. Suki, and K. R. Lutchen. Tidal stretches do not modulate responsiveness of intact airways in vitro. *J.Appl.Physiol* 2010; 109: 295-304.
20. Corsico, A., R. Pellegrino, M. C. Zoia, L. Barbano, V. Brusasco, and I. Cerveri. Effects of inhaled steroids on methacholine-induced bronchoconstriction and gas trapping in mild asthma. *Eur.Respir.J.* 2000; 15: 687-692.
21. Bel, E. H., M. C. Timmers, J. Hermans, J. H. Dijkman, and P. J. Sterk. The long-term effects of nedocromil sodium and beclomethasone dipropionate on bronchial responsiveness to methacholine in nonatopic asthmatic subjects. *Am.Rev.Respir.Dis.* 1990; 141: 21-28.
22. Sont, J. K., J. Han, J. M. van Krieken, C. E. Evertse, R. Hooijer, L. N. Willems, and P. J. Sterk. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. *Thorax* 1996; 51: 496-502.
23. Louis, R., L. C. Lau, A. O. Bron, A. C. Roldaan, M. Radermecker, and R. Djukanovic. The relationship between airways inflammation and asthma severity. *Am.J.Respir.Crit Care Med.* 2000; 161: 9-16.
24. Jensen, A., H. Atileh, B. Suki, E. P. Ingenito, and K. R. Lutchen. Selected contribution: airway caliber in healthy and asthmatic subjects: effects of bronchial challenge and deep inspirations. *J.Appl.Physiol* 2001; 91: 506-515.
25. Sont, J. K., P. Booms, E. H. Bel, J. P. Vandenbroucke, and P. J. Sterk. The severity of breathlessness during challenges with inhaled methacholine and hypertonic saline in atopic asthmatic subjects. The relationship with deep breath-induced bronchodilation. *Am.J.Respir.Crit Care Med.* 1995; 152: 38-44.
26. Chowdhury, B. A. and P. G. Dal. The FDA and safe use of long-acting beta-agonists in the treatment of asthma. *N.Engl.J.Med.* 2010; 362: 1169-1171.
27. Weatherall, M., M. Wijesinghe, K. Perrin, M. Harwood, and R. Beasley. Meta-analysis of the risk of mortality with salmeterol and the effect of concomitant inhaled corticosteroid therapy. *Thorax* 2010; 65: 39-43.
28. Gosens, R., I. S. Bos, J. Zaagsma, and H. Meurs. Protective effects of tiotropium bromide in the progression of airway smooth muscle remodeling. *Am.J.Respir.Crit Care Med.* 2005; 171: 1096-1102.
29. Bos, I. S., R. Gosens, A. B. Zuidhof, D. Schaafsma, A. J. Halayko, H. Meurs, and J. Zaagsma. Inhibition of allergen-induced airway remodelling by tiotropium and budesonide: a comparison. *Eur.Respir.J.* 2007; 30: 653-661.
30. Ayala, L. E. and T. Ahmed. Is there loss of protective muscarinic receptor mechanism in asthma? *Chest* 1989; 96: 1285-1291.
31. Shore, S. A., T. R. Bai, C. G. Wang, and J. G. Martin. Central and local cholinergic components of histamine-induced bronchoconstriction in dogs. *J.Appl.Physiol* 1985; 58: 443-451.
32. Kanazawa, H. Anticholinergic agents in asthma: chronic bronchodilator therapy, relief of acute severe asthma, reduction of chronic viral inflammation and prevention of airway remodeling. *Curr.Opin. Pulm.Med.* 2006; 12: 60-67.
33. Burns, G. P. and G. J. Gibson. A novel hypothesis to explain the bronchconstrictor effect of deep inspiration in asthma. *Thorax* 2002; 57: 116-119.
34. Legg, S. J. and C. O. Cruz. Effect of single and double strap backpacks on lung function. *Ergonomics* 2004; 47: 318-323.
35. Torchio, R., C. Gulotta, C. Ciacco, A. Perboni, M. Guglielmo, F. Crosa, M. Zerbini, V. Brusasco, R. E. Hyatt, and R. Pellegrino. Effects of chest wall strapping on mechanical response to methacholine in humans. *J.Appl.Physiol* 2006; 101: 430-438.
36. Torchio, R., C. Gulotta, P. Greco-Lucchina, A. Perboni, L. Montagna, M. Guglielmo, and J. Milic-Emili. Closing capacity and gas exchange in chronic heart failure. *Chest* 2006; 129: 1330-1336.
37. Holguin, F., S. Cribbs, A. M. Fitzpatrick, R. H. Ingram, Jr., and A. C. Jackson. A deep breath bronchoconstricts obese asthmatics. *J.Asthma* 2010; 47: 55-60.
38. Boulet, L. P., H. Turcotte, G. Boulet, B. Simard, and P. Robichaud. Deep inspiration avoidance and airway response to methacholine: Influence of body mass index. *Can.Respir.J.* 2005; 12: 371-376.

39. Mitzner, W. Airway smooth muscle: the appendix of the lung. *Am.J.Respir.Crit Care Med.* 2004; 169: 787-790.
40. Miller, J. D., G. Cox, L. Vincic, C. M. Lombard, B. E. Loomas, and C. J. Danek. A prospective feasibility study of bronchial thermoplasty in the human airway. *Chest* 2005; 127: 1999-2006.
41. Castro, M., A. I. Musani, M. L. Mayse, and N. S. Shargill. Bronchial thermoplasty: a novel technique in the treatment of severe asthma. *Ther.Adv.Respir.Dis.* 2010; 4: 101-116.
42. Castro, M., A. S. Rubin, M. Laviolette, J. Fiterman, L. M. De Andrade, P. L. Shah, E. Fiss, R. Olivenstein, N. C. Thomson, R. M. Niven, I. D. Pavord, M. Simoff, D. R. Duhamel, C. McEvoy, R. Barbers, N. H. ten Hacken, M. E. Wechsler, M. Holmes, M. J. Phillips, S. Erzurum, W. Lunn, E. Israel, N. Jarjour, M. Kraft, N. S. Shargill, J. Quiring, S. M. Berry, and G. Cox. Effectiveness and safety of bronchial thermoplasty in the treatment of severe asthma: a multicenter, randomized, double-blind, sham-controlled clinical trial. *Am.J.Respir. Crit Care Med.* 2010; 181: 116-124.
43. Cox, G., N. C. Thomson, A. S. Rubin, R. M. Niven, P. A. Corris, H. C. Siersted, R. Olivenstein, I. D. Pavord, D. McCormack, R. Chaudhuri, J. D. Miller, and M. Laviolette. Asthma control during the year after bronchial thermoplasty. *N.Engl.J.Med.* 2007; 356: 1327-1337.
44. Bel, E. H. Bronchial thermoplasty: has the promise been met? *Am.J.Respir.Crit Care Med.* 2010; 181: 101-102.
45. Cox, G., J. D. Miller, A. McWilliams, J. M. Fitzgerald, and S. Lam. Bronchial thermoplasty for asthma. *Am.J.Respir.Crit Care Med.* 2006; 173: 965-969.
46. Brown, R. H., W. Wizeman, C. Danek, and W. Mitzner. Effect of bronchial thermoplasty on airway distensibility. *Eur.Respir.J.* 2005; 26: 277-282.
47. Brown, R. H., W. Wizeman, C. Danek, and W. Mitzner. In vivo evaluation of the effectiveness of bronchial thermoplasty with computed tomography. *J.Appl.Physiol* 2005; 98: 1603-1606.
48. Auffray, C., I. M. Adcock, K. F. Chung, R. Djukanovic, C. Pison, and P. J. Sterk. An integrative systems biology approach to understanding pulmonary diseases. *Chest* 2010; 137: 1410-1416.
49. Kaminsky, D. A., C. G. Irvin, and P. J. Sterk. Complex systems in pulmonary medicine: a systems biology approach to lung disease. *J.Appl.Physiol* 2011; 110: 1716-1722.
50. Kitano, H. Systems biology: a brief overview. *Science* 2002; 295: 1662-1664.
51. Aderem, A. Systems biology: its practice and challenges. *Cell* 2005; 121: 511-513.
52. Kirschner, M. W. The meaning of systems biology. *Cell* 2005; 121: 503-504.
53. Frey, U., G. Maksym, and B. Suki. Temporal complexity in clinical manifestations of lung disease. *J.Appl. Physiol* 2011; 110: 1723-1731.
54. Frey, U. and B. Suki. Complexity of chronic asthma and chronic obstructive pulmonary disease: implications for risk assessment, and disease progression and control. *Lancet* 2008; 372: 1088-1099.