

available at www.sciencedirect.comjournal homepage: www.elsevier.com/locate/rmed

The effect of airway remodelling on airway hyper-responsiveness in asthma

Jessica A. Kermode^{a,b,d,*}, Nathan J. Brown^{a,b,d}, Kate M. Hardaker^{a,b,d},
 Claude S. Farah^{a,b,d}, Norbert Berend^{a,b,d}, Gregory G. King^{a,b,c,d},
 Cheryl M. Salome^{a,b,d}

^a The Woolcock Institute of Medical Research, PO Box M77, Missenden Road, Glebe, NSW 2050, Australia

^b Department of Medicine, The University of Sydney, NSW, Australia

^c Department of Respiratory Medicine, Royal North Shore Hospital, St Leonards, NSW, Australia

^d Co-operative Research Centre for Asthma and Airways, Australia

Received 17 December 2010; accepted 18 July 2011

KEYWORDS

Airway remodelling;
 Lung physiology;
 Physiology;
 Pulmonary function;
 Structure–function;
 Respiratory mechanics

Summary

Rationale: The mechanisms of airway hyper-responsiveness are only partially understood and the contribution of airway remodelling is unknown. Airway remodelling can be assessed by measuring airway distensibility, which is reduced in asthma, even when lung function is normal. We hypothesised that airway remodelling contributes to airway hyper-responsiveness in asthma, independent of steroid-responsive airway inflammation.

Objectives: To determine the relationship between airway distensibility and airway responsiveness at baseline and after 12 weeks of inhaled corticosteroid therapy in a group of asthmatics with airway hyper-responsiveness.

Methods: Nineteen doctor-diagnosed asthmatics had airway distensibility measured as the slope of the relationship between conductance and lung volume by the forced oscillation technique. Lung function, exhaled nitric oxide and methacholine challenge were also measured. Subjects had inhaled corticosteroid therapy for 12 weeks after which all measurements were repeated.

Results: At baseline, airway distensibility (mean, 95%CI) was 0.19(0.14–0.23) cm H₂O^{−1} s^{−1}, exhaled nitric oxide was 13.1(10.3–16.6) ppb and airway distensibility correlated with eNO ($p = 0.04$) and disease duration ($p = 0.02$) but not with airway responsiveness ($p = 0.46$), FEV₁ ($p = 0.09$) or age ($p = 0.23$). After treatment, exhaled nitric oxide decreased ($p = 0.0002$), FEV₁ improved ($p = 0.0001$), airway responsiveness improved ($p = 0.0002$), and there was a small improvement in airway distensibility but it did not normalise

* Corresponding author. Woolcock Institute of Medical Research, PO Box M77, Missenden Road, NSW 2050, Australia. Tel.: +61 2 9114 0146; fax: +61 2 9114 0014.

E-mail address: jesskermode@woolcock.org.au (J.A. Kermode).

($p = 0.05$). Airway distensibility was not correlated with either exhaled nitric oxide ($p = 0.49$) or airway responsiveness ($p = 0.20$).

Conclusions: Uncontrolled airway inflammation causes a small decrease in the distensibility of the airways of asthmatics with airway hyper-responsiveness. The lack of association between airway responsiveness and airway distensibility, both before and after 12 weeks ICS treatment, suggests that airway remodelling does not contribute to airway hyper-responsiveness in asthma.

© 2011 Elsevier Ltd. All rights reserved.

Introduction

The mechanisms of airway hyper-responsiveness (AHR), which is a distinguishing feature of asthma, are only partially understood. The airways in patients with asthma are characterised by the presence of airway inflammation and/or airway remodelling. Airway inflammation is clearly associated with AHR, since AHR correlates with markers of inflammation¹ and improves with anti-inflammatory treatment.^{2–6} The contribution of airway remodelling to AHR is unknown, yet is the subject of much debate, with current hypotheses ranging from airway remodelling enhancing airway responsiveness^{7–10} to airway remodelling protecting against AHR.^{9,11,12}

Airway remodelling can be assessed by measuring airway distensibility, which is reduced in asthma, even when lung function is normal,^{11,13–17} and is associated with thickening of the reticular basement membrane layer of the airway wall.¹⁶ The potential effects of remodelling on both the geometry (increased wall thickness) and mechanical properties (increased stiffness) of the airways are likely to result in irreversible airway narrowing and a reduction in the effects of deep inspirations, which modulate airway smooth muscle (ASM) contractility, with subsequent increases in AHR. An alternative proposal is that increased extracellular matrix and airway wall thickening could decrease airway compressibility, which may in turn be protective against AHR.⁹

To understand the contribution of airway remodelling to AHR the effects of inflammation on airway distensibility, the marker of remodelling, must be determined. The acute airway inflammatory response, consisting of an influx of inflammatory cells, mucus hypersecretion, and microvascular leakage leading to oedema,¹⁸ could thicken the airways thereby contributing to reduced airway distensibility. While short-term inhaled corticosteroid (ICS) treatment up to 12 weeks appears insufficient to alter remodelling, measured by reticular basement membrane thickness,⁶ it can effectively reduce both airway wall thickness, measured by high resolution computed tomography (HRCT),¹⁹ and inflammation, measured either by bronchiolar lavage,^{2,5} bronchial biopsy,^{2–4,6} or exhaled nitric oxide (eNO).⁴ The effects of ICS on markers of airway remodelling require longer-term treatment of 6–12 months.⁶ These differences in the time courses of response to ICS of the inflammatory and airway remodelling components of asthma can be exploited to determine the contribution of airway remodelling to AHR, since after treating steroid-responsive inflammation with relatively short-term ICS, any remaining association between airway

distensibility and AHR is likely to be due to the effects of airway remodelling.

We hypothesised that airway remodelling contributes to AHR in asthma, independent of steroid-responsive airway inflammation. Our specific aim was to determine the relationship between airway distensibility and airway responsiveness at baseline and after 12 weeks of inhaled corticosteroid therapy in a group of asthmatics with AHR.

Methods

Study subjects

Nineteen adults (9 males) with asthma were recruited by advertisements at The Woolcock Institute of Medical Research, The University of Sydney, and The Respiratory Clinic at Royal North Shore Hospital. Inclusion criteria were physician diagnosis of asthma plus either current symptoms or current treatment and airway hyper-responsiveness to methacholine. Subjects were excluded if they were current smokers, or had quit smoking within the previous six months, had a smoking history >10 pack years, had another respiratory or major illness, used oral prednisone or had a respiratory tract infection in the last month. All subjects provided written informed consent. The study was approved by the Human Ethics committee of the Sydney South West Area Health Service and the Northern Sydney Central Coast Health Service. The study was registered on the Australian Clinical Trials Registry (ACTRN12610000561088).

Study design

All subjects had a series of tests performed on a single visit to the laboratory after which they underwent 12 weeks of treatment with fluticasone propionate/salmeterol xinafoate 250/25 ug MDI 2 puffs bd via spacer. Following treatment, all measurements were repeated at a second visit. Short- and long-acting bronchodilator medications were withheld prior to each visit for at least 6 and 24 h, respectively. Asthma severity was based on the GINA guidelines.²⁰

Methods

Subjects completed a respiratory health questionnaire, the Asthma Control Questionnaire (ACQ),²¹ had allergen skin-prick tests, spirometry and whole body plethysmography using a Medisoft BodyBox 5500 (Medisoft Corporation, Sorries, Belgium). Exhaled nitric oxide (eNO) was measured

using an offline technique²² at 200 ml/s according to American Thoracic Society guidelines.²³

Airway distensibility was measured by the forced oscillation technique²⁴ and calculated as the slope of conductance (Grs) versus lung volume between 75% total lung capacity (TLC) and TLC. All subjects performed the following breathing protocol: 1 min of stable tidal breathing followed by a slow inhalation to TLC then another minute of tidal breathing. Subjects then inhaled slowly to TLC and breathed at approximately tidal volumes with progressively decreasing end-expiratory lung volumes until end expiration was near FRC, and tidal breathing was resumed. This deflation manoeuvre was performed twice after which the subject slowly inhaled to TLC to complete the test. Absolute lung volumes were calculated by referencing the maximal inhalations to plethysmographic TLC. Airway distensibility was calculated from Grs and lung volume data at points of zero flow (end inspiration and end expiration). The combined data from all deflation manoeuvres were used to plot Grs against lung volume and then the linear least-squares slope of Grs vs. lung volume between 75% TLC and TLC was calculated to give the index of airway distensibility. Although the previous study used two linear functions to describe the Grs–lung volume relationship: FRC to 75% TLC and 75% TLC to TLC, only the 75% TLC to TLC function discriminated between asthmatic and non-asthmatic subjects. Furthermore, airway distensibility between 75% TLC and TLC accounts for approximately 80% of total airway distensibility in both non-asthmatic and asthmatic subjects.²⁴ Therefore, in the current study, we only report airway distensibility between 75% TLC and TLC. Mean airway distensibility in non-asthmatic adults is 0.44 ± 0.08 .²⁴

Subjects had AHR measured by methacholine challenge²⁵ and defined as a $PD_{20}FEV_1 < 6.1 \mu\text{mol}$ methacholine. Responsiveness to methacholine was also measured on a continuous scale as the dose response slope (DRS).²⁶ The DRS was calculated as % fall in $FEV_1/\mu\text{mol}$ methacholine and a constant of 3 was added to allow for log transformation of zero or negative values. This allowed the inclusion of data from subjects who had a fall in FEV_1 of $<20\%$ either at baseline or after treatment. In terms of the continuous measure, AHR is defined as a DRS of $>3.27\%$ fall in $FEV_1/\mu\text{mol}$ methacholine, which is equivalent to a $PD_{20}FEV_1 < 6.1 \mu\text{mol}$ methacholine.

Analysis

Data were analysed using Analyse-It[®] for Microsoft Excel[®] software (Analyse-It Software Ltd, Leeds, UK) and the SAS[®] system (SAS Institute, Inc., Cary, NC). DRS and eNO were log-normally distributed and were log₁₀ transformed for all analyses. The relationships between AHR (expressed as DRS), inflammation (expressed as eNO) and airway distensibility were examined by performing univariate (Pearson) correlations. Multiple linear regression analyses were used to examine the predictors of baseline airway distensibility, airway distensibility after treatment, and the change in airway distensibility with treatment. The independent predictors included in the models were: FEV_1 (baseline, after treatment, or change in FEV_1 with treatment), eNO

(baseline, after treatment, or change in eNO with treatment) and ICS use, disease duration, age, height and weight. For categorical variables, data are expressed as number and percent of group, and analysed by the Chi-squared test. Data are shown as means with 95% confidence intervals (CI) unless otherwise specified. *p* Values < 0.05 were considered statistically significant.

Results

The individual characteristics of the subjects are shown in Table 1. The subjects were adults with mild- to moderate-persistent asthma,²⁰ and disease durations ranging from 1 to 53 years (Table 1). In these subjects airway distensibility was less than previously observed in non-asthmatics, and similar to that measured in mild to moderate asthmatics²⁴ (Table 1). Current inflammation was mild in this group, as shown by the relatively low eNO (Table 1).

At baseline, airway distensibility correlated with eNO ($r = 0.47$, $p = 0.04$) and disease duration ($r = -0.52$, $p = 0.02$) but not with DRS ($r = -0.18$, $p = 0.46$), FEV_1 ($r = 0.40$, $p = 0.09$) or age ($r = -0.29$, $p = 0.23$). Smoking history was negligible, as there were only 2 ex-smokers with 10 and 1.05 pack years, respectively.

Table 2 shows the effects of 12 weeks treatment with combination therapy on key outcome measures. As expected the treatment reduced eNO by 46% ($p = 0.0002$) and improved FEV_1 ($p = 0.0001$), DRS ($p < 0.0001$) and ACQ ($p = 0.0009$). There was a small improvement of $0.06 \text{ cm H}_2\text{O s}^{-1}$ ($p = 0.05$) in airway distensibility following treatment (see Fig. 1). After treatment, there were no correlations between airway distensibility and either eNO ($r = -0.18$, $p = 0.49$), DRS ($r = -0.31$, $p = 0.20$, see Fig. 2), FEV_1 ($r = 0.28$, $p = 0.24$) or age ($r = -0.14$, $p = 0.58$).

The multiple regression analyses confirmed these results. At baseline, eNO and duration of asthma were predictors of airway distensibility, independent of prior ICS use, FEV_1 , age height and weight (model $R^2 = 0.51$, $p = 0.004$). There were no independent predictors of airway distensibility after treatment or of the change in airway distensibility with treatment.

Discussion

In this study of patients with asthma, defined by the presence of airway hyper-responsiveness to methacholine, we found that airway distensibility correlated with eNO at baseline and that there was a small, borderline significant change in airway distensibility after 12 weeks of ICS treatment. These results suggest that steroid-responsive airway inflammation causes a small decrease in the distensibility of the airways of asthmatic subjects with AHR. After treatment, and despite the small improvement, airway distensibility remained abnormal and there was no correlation between airway distensibility and eNO, which suggests that the remaining abnormalities in airway distensibility are not due to steroid-responsive airway inflammation. These remaining abnormalities in airway distensibility are most likely due to airway remodelling. We also found that there was no correlation between airway

Table 1 Subject demographics at baseline.

Subject	Age, years	BMI, kg/m ²	Duration, years	FEV ₁ , % predicted	FEV ₁ /FVC	Airway distensibility, cm H ₂ O ⁻¹ s ⁻¹	PD ₂₀ FEV ₁ , μmol	ACQ	BDP equivalent dose, μg/day	eNO, ppb
1	55	20.2	34	67.07	0.81	0.03	0.60	2.57	1000	7.10
2	27	34.3	26	116.82	0.64	0.25	0.40	1.71	250	14.30
3	25	19.5	18	71.04	0.56	0.19	0.59	1.71	0	23.40
4	32	25.9	28	87.49	0.57	0.42	0.51	1.00	0	25.80
5	54	22.7	53	85.36	0.67	0.10	1.96	1.29	500	19.90
6	30	30.6	29	97.47	0.57	0.23	0.79	0.29	0	12.75
7	23	27.0	19	102.80	0.71	0.20	5.24	1.29	0	11.60
8	37	34.5	34	55.83	0.56	0.06	0.16	2.14	1000	6.00
9	26	22.8	16	99.95	0.68	0.22	0.78	0.71	0	24.85
10	46	29.1	36	63.41	0.51	0.12	0.37	2.14	500	21.25
11	40	30.6	39	81.46	0.52	0.10	0.13	2.29	500	7.55
12	20	22.4	17	109.85	0.86	0.28	5.08	0.00	0	22.35
13	29	27.3	24	73.07	0.62	0.35	0.21	1.71	0	23.10
14	59	25.4	34	78.75	0.54	0.09	0.47	0.85	0	7.00
15	23	22.4	20	91.32	0.79	0.14	1.18	1.14	250	7.05
16	35	23.4	30	73.55	0.54	0.16	0.14	0.57	0	10.70
17	62	27.4	1	79.41	0.68	0.33	0.89	0.71	250	6.40
18	24	22.6	23	95.80	0.66	0.08	0.15	1.57	0	18.25
19	45	25.8	10	84.76	0.48	0.20	0.27	1.00	0	11.90

Data are presented as mean ± 95% CI unless otherwise stated. FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; ppb, parts per billion; DRS, dose response slope; eNO, exhaled nitric oxide; ACQ, Asthma Control Questionnaire; BDP, beclomethasone dipropionate; BMI, body mass index; and PD₂₀FEV₁, provocative dose of methacholine inducing a 20% fall in FEV₁.

responsiveness and airway distensibility either before or after treatment, which suggests that airway remodelling does not contribute to AHR in asthma, independent of steroid-responsive airway inflammation.

We used an appropriate population of well-defined asthmatics, which allowed us to detect the effects of inflammation on all of the main outcome measures

including airway distensibility. The subjects in the study were selected because they had AHR and were likely to respond to anti-inflammatory treatment because of either poor ACQ or high eNO. The group also had reduced airway distensibility at baseline.²⁴ The baseline relationship between eNO and airway distensibility suggests that airway inflammation contributes to reduced airway distensibility,

Table 2 Subject characteristics.

Parameter	Baseline	After treatment	<i>p</i>
Age, years (range)	36.4 (20–62)		
BMI, kg/m ²	26.0 (24.0–28.0)		
Duration, years (range)	25.8 (1–53)		
FEV ₁ , % predicted	74.9 (67.2–82.6)	85.0 (77.8–92.3)	0.0001
FVC, % predicted	95.3 (88.3–102.3)	100.3 (93.7–107.0)	0.02
FEV ₁ /FVC	0.6 (0.6–0.7)	0.7 (0.6–0.7)	<0.0001
Airway distensibility ^a , cm H ₂ O ⁻¹ s ⁻¹	0.19 (0.14–0.23)	0.24 (0.20–0.29)	0.05
eNO ^b , ppb	13.1 (10.3–16.6)	7.1 (6.2–8.1)	0.0002
DRS ^b , % fall FEV ₁ /μmol methacholine	35.4 (22.9–54.8)	9.7 (6.1–15.4)	<0.0001
ACQ	1.3 (1.0–1.6)	0.7 (0.4–1.0)	0.0009
BDP equivalent dose, μg/day	236.1 (80.3–391.9)	2000	
ICS, % using	42.1	100	
RV, % predicted	147.8 (127.2–168.5)	129.1 (114.4–143.8)	0.04
FRC, % predicted	121.1 (109.0–133.2)	117.7 (107.5–127.8)	0.26
TLC, % predicted	115.5 (109.2–121.8)	114.6 (108.2–121.1)	0.25

Data are presented as mean ± 95% CI unless otherwise stated.

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; ppb, parts per billion; DRS, dose response slope; eNO, exhaled nitric oxide; ACQ, Asthma Control Questionnaire; FRC, functional residual capacity; ICS, patients on inhaled corticosteroid treatment; RV, residual volume; BDP, beclomethasone dipropionate; and BMI, body mass index.

^a Mean in normals = 0.44 ± 0.08.

^b Geometric mean.

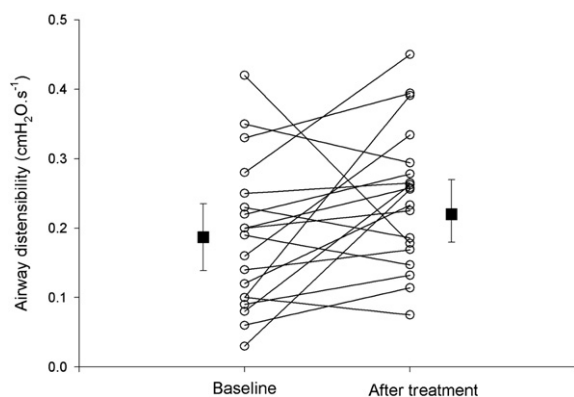


Figure 1 Airway distensibility was slightly altered after 12 weeks of ICS therapy in 19 treated asthmatics ($p = 0.05$). Closed squares are geometric means and 95% confidence intervals.

as measured by FOT, in asthma. Airway inflammation was measured indirectly using eNO, which is a well-established, non-invasive method. Although eNO correlates with eosinophilic inflammation^{27–29} and responds to ICS treatment,^{16,30} it was not possible to draw conclusions from the findings of the current study about the relationships between airway distensibility and specific inflammatory phenotypes.

We used treatment with ICS to determine if resolution of steroid-responsive inflammation altered airway distensibility. The treatment period of 12 weeks was expected to effectively resolve steroid-responsive inflammation⁶ but not to affect airway remodelling.^{5,6} The improvements in FEV₁, airway responsiveness, asthma control and eNO after treatment are consistent with this. However, the improvement in airway distensibility, which was of borderline significance, suggests that airway distensibility, measured by FOT, is partly influenced by steroid-responsive inflammation. The precise mechanism of this is unclear but it is likely that the steroid-responsive components of inflammation, including oedema and plasma exudate resulting from microvascular leakage,^{31,32} thicken the

airway walls thereby reducing airway distensibility. This is supported by the results of imaging studies in which wall thickness decreases with ICS.¹⁹ Alternatively there may be direct effects of the inflammatory milieu on airway mechanical properties.

It is important to note that despite the statistically significant improvement, airway distensibility remained abnormal after treatment. Furthermore, the mean ($\pm 95\%$ CI) absolute increase in airway distensibility was only 0.06 (± 0.05) $\text{cm H}_2\text{O s}^{-1}$, which is much less than the expected day to day variation of ± 0.203 $\text{cm H}_2\text{O s}^{-1}$.²⁴ Similarly, the changes for individual subjects were generally small, with only one subject having an increase and two subjects having decreases in airway distensibility that would be regarded as clinically meaningful, i.e. greater than ± 0.203 $\text{cm H}_2\text{O s}^{-1}$. These subjects had no systematic similarities or differences in prior treatment, duration of asthma, or any physiological characteristics that appear to explain their relatively large changes in airway distensibility with treatment. Since there was no correlation between eNO and airway distensibility after treatment, we propose that the remaining abnormalities in airway distensibility are not due to airway inflammation and are more likely due to the persistent effects of airway remodelling.

There are other factors that potentially contribute to reduced airway distensibility in asthmatics including increased ASM tone, reduced lung elastic recoil, long duration of disease and inflammation that is not responsive to inhaled corticosteroid therapy. However, differences in ASM tone and lung elastic recoil between asthmatics and non-asthmatics do not account for the differences in airway distensibility between the groups.²⁴ Duration of asthma has been associated with airway remodelling.³³ In our study, at baseline, duration of asthma was an independent predictor of airway distensibility and together with eNO accounted for 51% of the variance in airway distensibility. Although there were no significant independent predictors of airway distensibility after treatment, duration of asthma accounted for 19% of the variance in airway distensibility ($p = 0.06$). This is consistent with airway distensibility being a marker of airway remodelling. The possible effects of steroid resistant inflammation such as peribronchial inflammation on airway distensibility are unknown.

The lack of association between airway responsiveness and airway distensibility after short-term treatment suggests that airway remodelling is not an important determinant of AHR in asthma. Longer-term treatment studies have shown that the biggest improvements in AHR occur within the first few months with further incremental improvements for up to 18 months.^{34,35} It is generally assumed that the ongoing, long-term improvement in AHR is due to a gradual reduction in airway remodelling. The results of the current study do not contradict this hypothesis but suggest that any potential contribution of airway remodelling to AHR over the long term is likely to be small, and therefore large sample sizes will be required to detect any association.

We conclude that airway remodelling, as measured by reduced airway distensibility, is unlikely to have either a protective or detrimental effect on AHR in asthma.

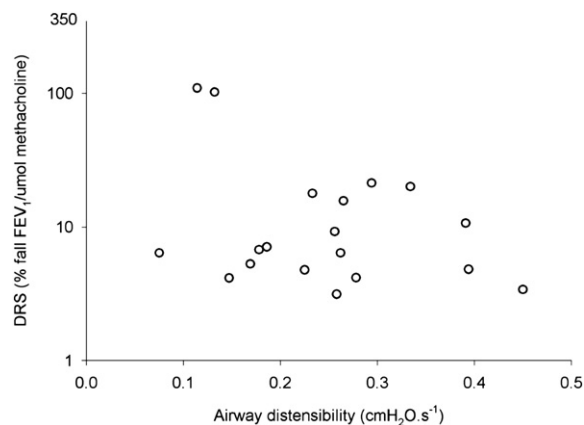


Figure 2 After treatment, airway distensibility and airway responsiveness were unrelated ($r = -0.31$, $p = 0.20$).

Acknowledgements

The authors would like to thank the volunteers who participated in this study; Gunnar Unger, Aaron Skelsey and Tom Li who gave engineering support for the exhaled nitric oxide and forced oscillation devices.

Funding

National Health and Medical Research Council (NHMRC) Australia (No: X08-0072) and Co-operative Research Council for Asthma and Airways, Australia.

Conflicts of interest

Ms Kermode, Dr Brown, Ms Hardaker, Prof Berend and Dr Salome have no conflicts of interest to disclose. Dr Farah has received honoraria from AstraZeneca and Novartis for educational meetings. Dr King has received various travel sponsorships from Boehringer Ingelheim, Pfizer, AstraZeneca and GlaxoSmithKline. The authors' affiliation, the Woolcock Institute of Medical Research, receives unrestricted grants from Boehringer Ingelheim, AstraZeneca and GlaxoSmithKline and has current and past consultancy agreements with Pfizer Inc, Boehringer Ingelheim, AstraZeneca and GlaxoSmithKline.

References

- Dupont LJ, Rochette F, Demedts MG, Verleden GM. Exhaled nitric oxide correlates with airway hyperresponsiveness in steroid-naïve patients with mild asthma. *Am J Respir Crit Care Med* 1998;157:894–8.
- Booth H, Richmond I, Ward C, Gardiner PV, Harkawat R, Walters EH. Effect of high-dose inhaled fluticasone propionate on airway inflammation in asthma. *Am J Respir Crit Care Med* 1995;152(1):45–52.
- Laitinen LA, Laitinen A, Haahtela T. A comparative-study of the effects of an inhaled corticosteroid, budesonide, and a beta-2-agonist, terbutaline, on airway inflammation in newly diagnosed asthma – a randomised, double-blind, parallel-group controlled trial. *J Allergy Clin Immunol* 1992;90(1):32–42.
- Pavord ID, Jeffery PK, Qiu Y, Zhu J, Parker D, Carlsheirner A, et al. Airway inflammation in patients with asthma with high-fixed or low-fixed plus as-needed budesonide/formoterol. *J Allergy Clin Immunol* 2009;123(5):1083–9.
- Trigg CJ, Manolitsas ND, Wang J, Calderon MA, McAulay A, Jordan SE, et al. Placebo-controlled immunopathologic study of four months of inhaled corticosteroids in asthma. *Am J Respir Crit Care Med* 1994;150(1):17–22.
- Ward C, Pais M, Bish R, Reid D, Feltis B, Johns D, et al. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. *Thorax* 2002;57(4):309–16.
- Boulet LP, Laviolette M, Turcotte H, Cartier A, Dugas M, Malo JL, et al. Bronchial subepithelial fibrosis correlates with airway responsiveness to methacholine. *Chest* 1997;112(1):45–52.
- Lambert RK, Wiggs BR, Kuwano K, Hogg JC, Pare PD. Functional significance of increased airway smooth muscle in asthma and COPD. *J Appl Physiol* 1993;74(6):2771–81.
- McParland BE, Macklem PT, Pare PD. Airway wall remodeling: friend or foe? *J Appl Physiol* 2003;95(1):426–34.
- Oliver MN, Fabry B, Marinkovic A, Mijailovich SM, Butler JP, Fredberg JJ. Airway hyperresponsiveness, remodeling, and smooth muscle mass – right answer, wrong reason? *Am J Respir Cell Mol Biol* 2007;37(3):264–72.
- Brackel HJ, Pedersen OF, Mulder PG, Overbeek SE, Kerrebijn KF, Bogaard JM. Central airways behave more stiffly during forced expiration in patients with asthma. *Am J Respir Crit Care Med* 2000;162(3 Pt 1):896–904.
- Niimi A, Matsumoto H, Takemura M, Ueda T, Chin K, Mishima M. Relationship of airway wall thickness to airway sensitivity and airway reactivity in asthma. *Am J Respir Crit Care Med* 2003;168(8):983–8.
- Colebatch HJ, Finucane KE, Smith MM. Pulmonary conductance and elastic recoil relationships in asthma and emphysema. *J Appl Physiol* 1973;34(2):143–53.
- Colebatch HJH, Greaves IA, Ng KY. Exponential analysis of elastic recoil and aging in healthy males and females. *J Appl Physiol* 1979;47(4):683–91.
- Johns DP, Wilson J, Harding R, Walters EH. Airway distensibility in healthy and asthmatic subjects: effect of lung volume history. *J Appl Physiol* 2000;88(4):1413–20.
- Ward C, Johns DP, Bish R, Pais M, Reid DW, Ingram C, et al. Reduced airway distensibility, fixed airflow limitation, and airway wall remodeling in asthma. *Am J Respir Crit Care Med* 2001;164(9):1718–21.
- Wilson JW, Li X, Pain MC. The lack of distensibility of asthmatic airways. *Am Rev Respir Dis* 1993;148(3):806–9.
- Scichilone N, Permutt S, Bellia V, Toghias A. Inhaled corticosteroids and the beneficial effect of deep inspiration in asthma. *Am J Respir Crit Care Med* 2005;172(6):693–9.
- Niimi A, Matsumoto H, Amitani R, Nakano Y, Sakai H, Takemura M, et al. Effect of short-term treatment with inhaled corticosteroid on airway wall thickening in asthma. *Am J Med* 2004;116(11):725–31.
- Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008;31(1):143–78.
- Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;14(4):902–7.
- Salome CM, Roberts AM, Brown NJ, Dermand J, Marks GB, Woolcock AJ. Exhaled nitric oxide measurements in a population sample of young adults. *Am J Respir Crit Care Med* 1999;159:911–6.
- American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171(8):912–30.
- Brown NJ, Salome CM, Berend N, Thorpe CW, King GG. Airway distensibility in adults with asthma and healthy adults, measured by forced oscillation technique. *Am J Respir Crit Care Med* 2007;176(2):129–37.
- Yan K, Salome C, Woolcock AJ. Rapid method for the measurement of bronchial responsiveness. *Thorax* 1983;38(10):760–5.
- O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose response curves to methacholine. An approach suitable for population studies. *Am Rev Respir Dis* 1987;136:1412–7.
- Berlyne GS, Parameswaran K, Kamada D, Efthimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. *J Allergy Clin Immunol* 2000;106(4):638–44.
- Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Pavord ID. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin Exp Allergy* 2005;35(9):1175–9.
- Warke TJ, Fitch PS, Brown V, Taylor R, Lyons JDM, Ennis M, et al. Exhaled nitric oxide correlates with airway eosinophils in childhood asthma. *Thorax* 2002;57(5):383–7.

30. van Rensen ELJ, Straathof KCM, Veselic-Charvat MA, Zwiderman AH, Bel EH, Sterk PJ. Effect of inhaled steroids on airway hyperresponsiveness, sputum eosinophils, and exhaled nitric oxide levels in patients with asthma. *Thorax* 1999;**54**(5): 403–8.
31. Greiff L, Andersson M, Erjefalt JS, Persson CGA, Wolmer P. Airway microvascular extravasation and luminal entry of plasma. *Clin Physiol Funct Imag* 2003;**23**(6):301–6.
32. Persson CGA. Role of plasma exudation in asthmatic airways. *Lancet* 1986;**2**(8516):1126–9.
33. Kurashima K, Kanauchi T, Hoshi T, Takaku Y, Ishiguro T, Takayanagi N, et al. Effect of early versus late intervention with inhaled corticosteroids on airway wall thickness in patients with asthma. *Respirology* 2008;**13**(7):1008–13.
34. Lundback B, Ronmark E, Lindberg A, Jonsson AC, Larsson LG, James M. Asthma control over 3 years in a real-life study. *Respir Med* 2009;**103**(3):348–55.
35. Reddel HK, Jenkins CR, Marks GB, Ware SI, Xuan W, Salome CM, et al. Optimal asthma control, starting with high doses of inhaled budesonide. *Eur Respir J* 2000;**16**(2):226–35.