

## Ventilation heterogeneity is a major determinant of airway hyperresponsiveness in asthma, independent of airway inflammation

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## **Abstract**

**Background:** Airway hyperresponsiveness is the ability of airways to narrow excessively in response to inhaled stimuli and is a key feature of asthma. Airway inflammation and ventilation heterogeneity have been separately shown to be associated with airway hyperresponsiveness.

**Objective:** To establish if ventilation heterogeneity was associated with airway hyperresponsiveness, independently of airway inflammation in asthmatics, and to determine the effect of inhaled corticosteroids on this relationship. **Methods:** In 40 asthmatic subjects, airway inflammation was measured by exhaled nitric oxide, ventilation heterogeneity by multiple breath nitrogen washout and airway hyperresponsiveness by methacholine challenge. In 18 of these subjects with uncontrolled symptoms, measurements were repeated after three months treatment with inhaled beclomethasone dipropionate. **Results:** At baseline, airway hyperresponsiveness was independently predicted by airway inflammation (partial  $r^2 = 0.20$ ,  $p < 0.001$ ) and ventilation heterogeneity (partial  $r^2 = 0.39$ ,  $p < 0.001$ ). Inhaled corticosteroid treatment decreased airway inflammation ( $p = 0.002$ ), ventilation heterogeneity ( $p = 0.009$ ), and airway hyperresponsiveness ( $p < 0.001$ ). After treatment, ventilation heterogeneity was the sole predictor of airway hyperresponsiveness ( $r^2 = 0.64$ ,  $p < 0.001$ ). **Conclusion:** Baseline ventilation heterogeneity is a strong predictor of airway hyperresponsiveness, independent of airway inflammation in asthmatic subjects. Its persistent relationship with airway hyperresponsiveness following anti-inflammatory treatment suggests that it is an important independent determinant of airway hyperresponsiveness. **Clinical implication:** The strength of the association between ventilation heterogeneity and airway hyperresponsiveness elucidates a possible mechanism of airway hyperresponsiveness in asthma. Consequently, the normalisation of ventilation heterogeneity is a potential goal of therapy that may lead to improved long term outcomes.

## **Introduction**

Airway hyperresponsiveness (AHR) is a common feature of asthma that is defined as the ability of airways to narrow too easily and by too much in response to provoking stimuli. In asthmatic subjects with severe AHR, excessive airway narrowing represents the potential for severe and life-threatening asthma attacks. (1) AHR is a clinically useful tool for treatment monitoring, (2) and is associated with impaired development of lung function in childhood. (3) Although AHR has been shown to be associated with airway inflammation, (4) and ventilation heterogeneity, (5) the mechanisms that cause AHR are poorly understood.

Although airway inflammation is regarded as the underlying cause of AHR (6), it is not known exactly how inflammation may cause AHR. Significant associations between AHR and markers of eosinophilic inflammation in induced sputum, (7) and the level of nitric oxide in exhaled breath (4) have been observed, however there are also contradictory studies which fail to show any association between airway inflammation and AHR (8) (9). Evidence suggests that other non-eosinophilic pathways such as neutrophilic inflammation are important contributors to AHR and asthma. (10) Exhaled nitric oxide is an indirect marker of eosinophilic airway inflammation that is easy to perform, is reduced with anti-inflammatory inhaled corticosteroid treatment (ICS), (2) and is more closely associated with AHR and active asthma than other markers of airway inflammation such as sputum eosinophils (4) and serum markers. (11) For these reasons, exhaled nitric oxide may be a suitable marker of eosinophilic inflammation to explore the relationship between AHR, airway inflammation and ventilation heterogeneity, and to determine the effect of ICS treatment on this relationship.

Ventilation heterogeneity is another potential cause of AHR. (5) Supportive evidence for a causal association arises from computer simulations whereby a more uneven distribution of ventilation and airway narrowing throughout the lung results in a greater overall increase in airway resistance following bronchoconstriction (ie. AHR). (12) (13) (14) Ventilation heterogeneity can be assessed non-invasively by the multiple breath nitrogen washout (MBNW). (15) The greater the degree of ventilation heterogeneity within the lung during continuous tidal breathing of 100% oxygen, the greater the nitrogen phase III slope in each subsequent MBNW expiration. More specifically, phase III slope is determined by convective gas transport, predominantly in the conductive airways, and by diffusive transport in the more peripheral acinar airways. From multiple breath washout tests using inert gases with different diffusivities (16), SF<sub>6</sub> and helium, it has been possible to partition the convection from the diffusion-dependent portion of the phase III slope, and derive corresponding indices of conductive airway heterogeneity ( $S_{cond}$ ) and acinar heterogeneity ( $S_{acin}$ ). The value of these indices increases as ventilation heterogeneity in the corresponding lung zone increases. The mathematical derivation of these indices is explained in the supplementary data. Using this MBNW analysis, ventilation heterogeneity in conductive and acinar lung zones have been shown to be abnormal in asthma (17) and to be more sensitive than spirometry for detecting early changes to the peripheral airways associated with cigarette smoking. (15)

In the present study, we hypothesised that there would be an association between ventilation heterogeneity and AHR, and we tested whether this association was independent of airway inflammation in a cross-sectional study of asthmatic subjects with a wide range of severity. In a sub-group of these asthmatic subjects with clinically uncontrolled asthma, we further tested the

relationship between airway inflammation, ventilation heterogeneity and AHR following 3 months of treatment with inhaled corticosteroids.

## **Methods**

### **Study design**

We enrolled subjects who had asthma as diagnosed by a respiratory physician, according to the NIH guidelines. (18) At the baseline visit, all subjects underwent these tests in the following order: 1) atopic status was determined by skin prick test with mean wheal diameters  $\geq 4$  mm regarded as positive, 2) juniper questionnaire, 3) fraction of nitric oxide in exhaled breath ( $F_{ENO}$ ) was used as a marker of airway inflammation, 4) MBNW was performed to measure of ventilation heterogeneity and lastly, 5) methacholine challenge was undertaken to determine AHR. A sub-group with at least mild persistent asthma, as determined by GINA guidelines, (19) were given inhaled corticosteroid treatment for three months with either chlorofluorocarbon beclomethasone dipropionate (CFC-BDP) 750 $\mu$ g bd via autohaler or hydrofluoroalkane beclomethasone dipropionate (HFA-BDP) 400 $\mu$ g bd via autohaler, being clinically equivalent doses. Since our aim was to investigate overall treatment effects, all treatment data were pooled for analyses. Written informed consent was obtained from all subjects and the study was approved by the Human Ethics Review Committee of the South-Western Area Health Service. This study is registered on the Australian Clinical Trials Registry (#ACTRN012605000317695).

### **Subjects**

Asthmatic subjects were recruited by advertising throughout the University of Sydney. Inclusion criteria for all asthmatics were: 1) no smoking within the last six months and less than a 10 pack years smoking history, 2) no current lung disease other than asthma, 3) no oral prednisone use in the last four weeks, and 4) no respiratory tract infection in the last four weeks. Additional inclusion criteria for entry into the three month treatment period were: 1) having asthma symptoms on at least three occasions a week over the previous month, 2) having AHR defined as a provoking dose of methacholine causing a 20% fall in  $FEV_1$  ( $PD_{20}FEV_1$ ) of 6.1 $\mu$ mol or less, and 3) taking no more than 800 $\mu$ g/day chlorofluorocarbon-beclomethasone dipropionate equivalent of inhaled corticosteroids.

Two cohorts of healthy non-asthmatic subjects were recruited from our research staff and the University of Sydney. The first cohort was recruited for the sensitivity and specificity analysis of a parameter from the MBNW test in detecting AHR, and the second cohort was recruited for testing the repeatability of the MBNW indices. Healthy non-asthmatic subjects were eligible to participate if they had 1) no history of, or medication use relating to chronic respiratory disease, 2) no smoking within the last six months and less than a 10 pack years smoking history, and 3) no respiratory tract infection in the last four weeks.

### **Symptoms**

All asthmatic subjects completed the juniper asthma control questionnaire (20) at the baseline visit and after 3 months of treatment (in the treatment sub-group) to assess asthma symptoms within the last one week.

### **Exhaled Nitric Oxide**

The fraction of exhaled nitric oxide ( $F_{ENO}$ ) was measured using an offline technique (21) according to American Thoracic Society guidelines. The subject exhaled over 5-15 seconds into a nitric oxide impermeable polyethylene bag (Scholle Industries Pty Ltd, Elizabeth West, Australia), at a constant flow of 0.2L/sec, monitored by rotameter (Dwyer Flowmeter Model

VFASS-25, AMBIT Instruments Pty Ltd, Parramatta, Australia). The exhaled gas was analysed using a chemiluminescence analyser (Thermo Environmental Instruments Model 42C). The upper limit of normal for  $F_{ENO}$  has been established as 13 parts per billion (ppb). (22)

### Multiple Breath Nitrogen Washout

The MBNW was performed using a closed circuit, bag-in-box breathing system to deliver 100%  $O_2$  during inspiration with separate capture of exhaled breath. Nitrogen ( $N_2$ ) concentration was measured at the mouth by a Model 721 KaeTech Nitrogen Analyser (KaeTech Instruments Inc, Green Bay, WI, USA), while flow and volume were recorded from the box by a pneumotachograph. Subjects breathed 100%  $O_2$  at a tidal volume of between 1 and 1.3 litres, until mean expired  $N_2$  concentration dropped to  $<2\%$ . Three tests were performed with successive tests starting after alveolar  $N_2$  had returned to baseline. Ventilation heterogeneity indices in the conductive ( $S_{cond}$ ) and gas exchanging ( $S_{acin}$ ) lung zones, were derived as previously described (17) (see supplementary data for calculations). The upper limit of normal for  $S_{cond}$  was  $0.037 L^{-1}$  and for  $S_{acin}$  was  $0.130 L^{-1}$ , derived from the mean +  $1.96 \times SD$  of the value obtained previously in normal subjects. (23) The lung clearance index (LCI) was calculated by dividing the cumulative expired volume (CEV) during the washout, by the patient's functional residual capacity (FRC) determined from the washout (ie.  $LCI = CEV/FRC$ ). Details of a repeatability study for  $S_{cond}$ ,  $S_{acin}$  and LCI obtained from an independent asthmatic and healthy non-asthmatic patient group, can be found in the supplementary data.

### Spirometry and bronchial challenge

Spirometry was performed using a SensorMedics Vmax spirometer (SensorMedics Corporation, Yorba Linda CA, USA), and methacholine challenge tests were performed using the rapid method (24) via hand-held De Vilbiss N° 45 nebulisers in which doubling doses, ranging from  $0.05 \mu mol$  to  $6.1 \mu mol$ , were administered until the final dose was reached or the  $FEV_1$  fell by  $\geq 20\%$ . Short acting  $\beta$ -agonists were withheld for six hours and long-acting  $\beta$ -agonists for 24 hours before testing. Response to challenge was measured by the dose response ratio (DRR), which is a continuous variable based on the two-point slope (25). DRR is calculated from the final step in the challenge test, as  $\% fall FEV_1 / \text{methacholine dose } (\mu mol)$ , and a constant of 3 is added to allow for log transformation of zero or negative values. Greater values of DRR indicate greater AHR, and a DRR value of greater than  $6.28 \% fall in FEV_1 / \mu mol \text{ methacholine} + 3$ , indicates the presence of AHR. This allows inclusion of data from subjects who had a fall in  $FEV_1$  less than 20% either at baseline, or following treatment.

### Statistical Analysis

Data were analysed using the Analyse-it for Microsoft Excel (Analyse-It Software Ltd, Leeds, England) software. DRR and  $F_{ENO}$  were log-normally distributed and were  $\log_{10}$  transformed for all analyses. Change in DRR after treatment was expressed as change in doubling dose of methacholine (change in  $\log DRR / 0.3$ ). The relationship between AHR (expressed as DRR) and potential predictive factors was examined using univariate analysis (using Pearson's correlation coefficient) and using multiple linear regression analyses. The sensitivity and specificity of  $S_{cond}$  in predicting AHR was assessed by Receiver Operator Characteristics (ROC) analysis. The paired student's t-test was used for parametric data, and Wilcoxon signed rank test for non-parametric data to compare outcomes after treatment. Data are presented as mean  $\pm 95\%$  confidence intervals unless otherwise specified.

## **Results**

### **Subjects**

40 asthmatic subjects participated in the baseline study. 28 of these subjects had sub-optimally controlled asthma and were eligible for the three-month treatment study, and 24 agreed to participate. Four subjects from the treatment sub-group withdrew from the study during treatment, and two subjects' results were excluded from the analysis due to an upper respiratory tract infection at their post-treatment visit. Therefore, 18 subjects were included in our treatment sub-group analysis.

### **Baseline results of entire asthma group**

Characteristics of all 40 asthmatics at baseline are shown in Table 1.

**Table 1:** Subject characteristics. Values are means and (SD) unless otherwise stated.

	Entire asthma group (n=40)	ICS treatment asthma sub-group (n=18)			p <sup>†</sup>
		Before treatment	After treatment	Abs Diff <sup>†</sup> (95% CI)	
<b>M/F</b>	20/20	9/9	-	-	-
<b>Age (years) and (range)</b>	32.1 (18-66)	33.9 (18-63)	-	-	-
<b>Atopic Y/N</b>	40/0	-	-	-	-
<b>FE<sub>NO</sub><sup>*</sup> (ppb)</b>	15.4 (12.2-19.4)	14.1	8.0	6.5 (1.5-11.6)	0.002
<b>S<sub>cond</sub> (L<sup>-1</sup>)</b>	0.055 (0.03)	0.067	0.052	0.015 (0.01-0.03)	0.009
<b>S<sub>acin</sub> (L<sup>-1</sup>)</b>	0.146 (0.02)	0.159	0.142	0.017 (0.00-0.03)	0.035
<b>LCI (CEV/FRC)</b>	8.16 (1.1)	8.54	7.78	0.77 (0.4-1.1)	0.001
<b>DRR (% fallFEV<sub>1</sub>/μmol methacholine+3)<sup>*</sup></b>	18.9 (12.4 – 28.8)	22.0	10.1	1.1 <sup>§</sup> (0.6-1.6)	<0.001
<b>FEV<sub>1</sub> (% predicted)</b>	82.4 (11.7)	80.1	85.9	5.8 (3.2-8.4)	<0.001
<b>FEV<sub>1</sub>/FVC ratio</b>	0.76 (0.08)	0.73	0.75	0.02 (0.00-0.04)	0.04
<b>β-agonist use (times/day)</b>	1.4 (2.4)	1.95	0.54	0.82 (-0.0-1.6)	0.07
<b>BDP equiv. dose (μg/day)</b>	320 (449.6)	270	1500	-	-
<b>Juniper symptom score</b>	1.28 (0.84)	1.48	0.81	0.65 (0.4 - 0.9)	<0.001

\* = Geometric mean and 95% CI

† = Comparison between values before and after treatment

ppb = parts per billion

BDP = Beclomethasone Dipropionate

equiv. = equivalent

§ = doubling dose difference in DRR

AHR was present in 31/40 subjects, and logDRR correlated positively with airway inflammation ( $\log F_{\text{ENO}}$ ), ventilation heterogeneity in conducting airways ( $S_{\text{cond}}$ ) (Figure 1a), lung clearance index (LCI) and negatively with percent predicted  $FEV_1$  and  $FEV_1/FVC$  ratio (Table 2).

**Table 2:** Pearson correlation coefficients of baseline variables with AHR (dose response ratio) for the asthmatic group as a whole and for the treatment sub-group before and after treatment.

	<b>Baseline asthma group (n=40)</b>		<b>Baseline Treatment sub-group (n=18)</b>		<b>Post treatment Treatment sub-group (n=18)</b>	
	Pearsons r	p value	Pearsons r	p value	Pearsons r	p value
Airway inflammation (F <sub>E</sub> NO)	0.62	< 0.001	0.51	0.03	0.61	0.008
Ventilation heterogeneity (S <sub>cond</sub> )	0.63	< 0.001	0.64	0.004	0.82	<0.001
Ventilation heterogeneity (S <sub>acin</sub> )	0.07	0.67	0.04	0.86	-0.02	0.93
Ventilation heterogeneity (LCI)	0.38	0.02	0.31	0.21	0.47	0.05
FEV <sub>1</sub> % predicted	-0.44	0.005	-0.40	0.10	-0.37	0.13
FEV <sub>1</sub> /FVC ratio	-0.47	0.002	-0.34	0.17	-0.21	0.41

Log DRR was not correlated with  $S_{acin}$  ( $p = 0.67$ ) . Log  $F_{ENO}$  was not correlated with  $S_{cond}$  ( $r = 0.29$ ,  $p = 0.07$ ),  $S_{acin}$  ( $r = 0.20$ ,  $p = 0.22$ ) or LCI ( $r = 0.14$ ,  $p = 0.40$ ) at baseline.

In a multiple linear regression model which included the significant factors in Table 2, log DRR was significantly predicted by both log  $F_{ENO}$  (partial  $r^2 = 0.20$ ,  $p < 0.001$ ,  $\beta$  coefficient = 0.9, 95% CI = 0.5 to 1.3) and  $S_{cond}$  (partial  $r^2 = 0.39$ ,  $p < 0.001$ ,  $\beta$  coefficient = 8.5, 95% CI = 4.8 to 12.2). LCI, percent predicted  $FEV_1$  and  $FEV_1/FVC$  ratio were not significant predictors of log DRR ( $p = 0.90$ ,  $p = 0.25$  and  $p = 0.97$ , respectively) when included separately, or together in this model.

### **Inhaled corticosteroid treatment sub-group**

Characteristics of the 18 asthmatic subjects in the treatment sub-group are shown in Table 1. Following treatment, there were significant improvements in DRR,  $F_{ENO}$ ,  $S_{cond}$ ,  $S_{acin}$ , LCI,  $FEV_1$  (% predicted) and the juniper symptom score (Table 1), but AHR was still present in 10/18 subjects. The univariate correlations at baseline in this sub-group were similar to those in the group as a whole (Table 2, Figure 1b). However, LCI, percent predicted  $FEV_1$  and  $FEV_1/FVC$  ratio were not correlated with log DRR either before or after treatment in the sub-group (Table 2).

In a multiple linear regression model, log DRR in the treatment sub-group was predicted solely by  $S_{cond}$  both before ( $r^2 = 0.38$ ,  $p = 0.004$ ,  $\beta$  coefficient = 8.3, 95% CI = 3.1 to 13.6) and after treatment ( $r^2 = 0.64$ ,  $p < 0.001$ ,  $\beta$  coefficient = 10.9, 95% CI = 6.8 to 15). Log  $F_{ENO}$ , LCI, percent predicted  $FEV_1$  and  $FEV_1/FVC$  ratio were not significant predictors of log DRR either before ( $p = 0.18$ ,  $p = 0.90$ ,  $p = 0.24$ ,  $p = 0.99$ , respectively) or after treatment ( $p = 0.38$ ,  $p = 0.30$ ,  $p = 0.25$ ,  $p = 0.42$ , respectively) in the multivariate regression model.

The magnitude of improvement in AHR correlated with both the reductions in  $F_{ENO}$  and  $S_{cond}$  ( $F = 3.94$ ,  $p = 0.04$  for the multivariate model), however there was no correlation between the reductions in  $S_{cond}$  and  $F_{ENO_{0.2}}$  following treatment ( $r = 0.07$ ,  $p = 0.77$ ). There were no differences in the magnitude of improvements in  $F_{ENO}$  ( $p = 0.26$ ),  $S_{cond}$  ( $p = 0.81$ ), or DRR ( $p = 0.90$ ) between subjects treated with HFA-BDP or CFC-BDP (corresponding post-treatment data are represented by squares and triangles in Figure 1b).

### **$S_{cond}$ as a predictor of AHR**

Based on the correlation between AHR and  $S_{cond}$ , we combined the asthmatic cohort from this study ( $n = 40$ ) and a cohort of 17 healthy non-asthmatic subjects (their characteristics are summarised in table E1 in the supplementary data), to test whether  $S_{cond}$  was a significant predictor of the presence of AHR, using receiver operator characteristics (ROC) analysis (Figure 2). The area under the ROC curve was 0.88 ( $p < 0.0001$ ), and the cut-off value of  $S_{cond} = 0.037 L^{-1}$  corresponding to the upper limit of normal range (26) gave a good combination of sensitivity and specificity, (71.9% and 90.0%, respectively) for detecting the presence of AHR. Using the same  $0.037 L^{-1}$  cut-off value,  $S_{cond}$  remained a significant predictor of the presence of AHR after treatment in our 18 subjects from the sub-group, (area under the ROC curve = 0.96,  $p < 0.0001$ ) with a high sensitivity and specificity (90.0% and 87.5%, respectively, see figure E2 in the supplementary data).

### **Repeatability of $S_{cond}$ , $S_{acin}$ and LCI**

Characteristics of the cohort of 10 asthmatic and 11 healthy non-asthmatic subjects who participated in the repeatability study of the MBNW indices are summarised in the supplementary data (table E2). In asthmatic subjects,  $S_{\text{cond}}$ ,  $S_{\text{acin}}$  and LCI had a good repeatability with the intra-class correlation coefficient (ICC) for  $S_{\text{cond}} = 0.84$  (95% limits of agreement =  $\pm 0.026$ ), the ICC for  $S_{\text{acin}} = 0.95$  (95% limits of agreement =  $\pm 0.027$ ), and the ICC for LCI = 0.91 (95% limits of agreement =  $\pm 0.735$ ). For the repeatability results in the healthy non-asthmatic subjects, refer to table E3 in the supplementary data.

## **Discussion**

In this study, we have shown that baseline ventilation heterogeneity correlates strongly with the severity of AHR in asthmatic subjects with a range of disease severity. In particular, conductive ventilation heterogeneity ( $S_{\text{cond}}$ ), predicted AHR to methacholine independently of airway inflammation and airway calibre. Importantly, the strong correlation between  $S_{\text{cond}}$  and AHR persisted after airway inflammation was reduced with ICS treatment. Following treatment,  $S_{\text{cond}}$  was, in fact, the sole independent predictor of AHR. The improvement in  $S_{\text{cond}}$  with ICS treatment suggests that it is determined, at least in part, by steroid-responsive inflammatory processes. However, the residual ventilation heterogeneity which persisted after ICS treatment suggests that non-steroid responsive or non-inflammatory factors also make an important contribution to ventilation heterogeneity. The conductive airways, where the residual ventilation heterogeneity responsible for AHR after anti-inflammatory treatment originates, clearly constitute an important therapeutic target. These findings are novel and have clinical implications in patients who have asthma.

Ventilation heterogeneity in the lungs is a well recognised feature in asthma (12) but its clinical significance, apart from the deleterious effects on gas exchange (27), has been uncertain. In our study, the main contribution to ventilation heterogeneity that was associated with AHR, originated in the conductive airways. Gustafsson et al (5) reported a correlation between airway responsiveness to cold air challenge and ventilation heterogeneity measured by single breath washout using helium and SF<sub>6</sub>, which indicated an involvement of peripheral airways, but did not assess the contribution of airway inflammation. It is impossible to independently determine conductive airways ventilation heterogeneity from the single breath washout, making comparison with the present study difficult. However, despite methodological differences in the determination of ventilation heterogeneity and AHR, the observed correlations between ventilation heterogeneity and AHR in both studies suggest a robust association. In the present study, AHR was not correlated with baseline acinar ( $S_{\text{acin}}$ ) ventilation heterogeneity, signalling that the important lung region with regards to the relationship between ventilation heterogeneity and AHR is specifically in the conducting airways. The weak correlation of the lung clearance index (LCI) with AHR indicates that this measure of specific ventilation distribution between relatively large lung units, which also partly contributes to  $S_{\text{cond}}$ , is neither sensitive nor specific as a measure of the conductive airway heterogeneity underlying AHR.

The significant reduction in ventilation heterogeneity in our subjects following anti-inflammatory ICS treatment suggests that ventilation heterogeneity is partly due to steroid-responsive airway inflammation. After treatment, residual ventilation heterogeneity persisted in the presence of normalised mean exhaled nitric oxide levels, suggesting that other inflammatory processes not related to exhaled nitric oxide, or non-inflammatory processes could also contribute to ventilation heterogeneity. Exhaled nitric oxide correlates with sputum eosinophils (28), however it does not reflect the full spectrum of inflammatory and non-inflammatory processes associated with asthma. This may explain the lack of association between improvement in exhaled nitric oxide and ventilation heterogeneity during treatment. Ventilation heterogeneity could also continue to improve with further ICS treatment, as has been shown to occur with AHR, which can continue to improve up to and after 12 months of treatment. (29) It would be valuable in future studies to also assess any additional benefits of systemic treatments to ventilation heterogeneity.

The residual ventilation heterogeneity present at the end of treatment may also be due to non-inflammatory structural changes, such as airway remodelling or elevated smooth muscle tone. Structural changes induced by airway remodelling are likely to be heterogeneously distributed amongst parallel pathways of the bronchial tree. These structural changes manifest as altered composition and organisation of the soft tissues of the airway walls, and are believed to be a result of chronic or repeated episodes of acute inflammation (30). The changes include thickening of the airway wall, smooth muscle hyperplasia and hypertrophy, subepithelial fibrosis, and mucous metaplasia. It is reasonable that many of these abnormalities, if not all, contribute to ventilation heterogeneity by exaggerating the inherent heterogeneity of the airway tree, and by being unevenly distributed themselves.

It is possible that increased baseline ventilation heterogeneity caused a heterogeneous distribution of methacholine aerosol, resulting in high concentrations of methacholine delivered to a small surface area of the airways. In a previous study, central-to-peripheral airway differences in the pattern of methacholine deposition in asthmatic subjects did not alter AHR (31). However, a recent imaging study in sheep (32) demonstrated that regions of lung with the highest baseline ventilation were the same regions which became constricted after methacholine, suggesting a link between parallel ventilation heterogeneity and methacholine deposition. This could partly explain the association between  $S_{\text{cond}}$  (representative of parallel ventilation heterogeneity) and AHR. The exact mechanism for how ventilation heterogeneity may cause AHR remains unclear. However, a recent modelling study (12) demonstrated that only minor alterations in structure, function or smooth muscle activity in a virtually uniform airway tree are required to trigger extreme ventilation heterogeneity during uniformly applied bronchoconstriction.

The strengths of our study were the wide range of asthma severity in the group of subjects tested at baseline. Indices of ventilation heterogeneity from the MBNW were robust, repeatable and comparable to values obtained in other asthmatics. (17, 33) The strong predictive capacity of  $S_{\text{cond}}$  for the presence of AHR, as inferred from our ROC analysis (figure 2), is reinforced because the upper limit of the normal range for  $S_{\text{cond}}$  ( $0.037 \text{ L}^{-1}$ ), corresponded to a high combination of sensitivity and specificity for detecting the presence of AHR in the present study. This raises the possibility that  $S_{\text{cond}}$  could at least partly be used to predict AHR as part of the clinical management of asthma. MBNW tests can be performed in patients whose lung function is too low to permit bronchial challenge, and has been successfully administered in un-sedated children with a number of airway diseases. (34)

In summary, we have shown for the first time that ventilation heterogeneity in the conducting airways is a significant predictor of AHR in asthmatic subjects, independent of airway inflammation as measured by exhaled nitric oxide, both before and after ICS treatment. Inflammatory and non-inflammatory processes which could alter the structural features within the airway tree may contribute to ventilation heterogeneity. Based on the strength of the correlations in this study and on the results of computational modelling (12) (13) (14), it is possible that ventilation heterogeneity is an important contributor to AHR in asthma. The index  $S_{\text{cond}}$  derived from the MBNW is a measure of conducting airway function that is likely to be useful in the clinical management of asthma and for the assessment of new treatment strategies

and drugs in this disease. These findings suggest that greater understanding of the determinants of ventilation heterogeneity may lead to more efficacious asthma treatment and can provide an additional tool for treatment monitoring, resulting in improved long term outcomes for asthmatic patients.

### **Figure legends**

**Figure 1: a)** Conductive airway ventilation heterogeneity ( $S_{\text{cond}}$ ) correlates with AHR (logDRR) at baseline ( $r = 0.63$ ,  $p < 0.001$ ). Closed circles (●) are those who participated in ICS treatment study and open circles (○) are those who did not. **b)**  $S_{\text{cond}}$  continues to correlate with AHR following treatment ( $r = 0.79$ ,  $p < 0.001$ ). Triangles (▲) are the CFC-BDP treated and squares (■) are the HFA-BDP treated subjects.

**Figure 2:** Sensitivity and specificity of  $S_{\text{cond}}$  in predicting the presence or absence of AHR in a combination of 40 asthmatics and 17 healthy non-asthmatic subjects at baseline

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### **Competing interests:**

These authors have the following potential conflicts of interest to declare:

#### **Cheryl M. Salome:**

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#### **Norbert Berend:**

"N.Berend serves on advisory boards for GlaxoSmithKline, Boehringer Ingelheim and AstraZeneca. The Woolcock Institute of Medical Research of which N.Berend is the Director receives research grants from GlaxoSmithKline."

#### **Gregory G. King:**

"Gregory King has received travel sponsorship from GlaxoSmithKline to attend the ATS ASM 2003 (approximately \$AUS9,000) and a GlaxoSmithKline meeting in 2003 (approximately \$AUS10,000), travel sponsorship from AstraZeneca to attend ATS ASM 2004 (approximately \$AUS10,000) and 2 AstraZeneca scientific meetings (approximate combined value \$20,000), an honorarium paid to his research institute from GlaxoSmithKline to speak at a sponsored conference in South East Asia (\$AUS3,000) in 2005, a travel grant from AstraZeneca to attend the ATS 2005 (\$AUS6,000) and a travel grant from GlaxoSmithKline to attend the ATS 2006 (\$AUS5,000). A proportion of Dr King's research work is conducted at the Woolcock Institute of Medical Research, which receives unrestricted grants from AstraZeneca, GlaxoSmithKline, Boehringer Ingelheim and Vita Medical for such work. The Woolcock Institute of Medical Research also has a consultancy agreement with Pfizer, Boehringer Ingelheim, AstraZeneca and GlaxoSmithKline for which Dr King provides consultancy services related to asthma and COPD. His research group receives a proportion of the grants and monies that arise from those companies, as part of specific and general allocations of those funds for research purposes across all research groups of the Woolcock Institute of Medical Research. Dr King does not own any stocks, equity or patents that pose a conflict of interest."

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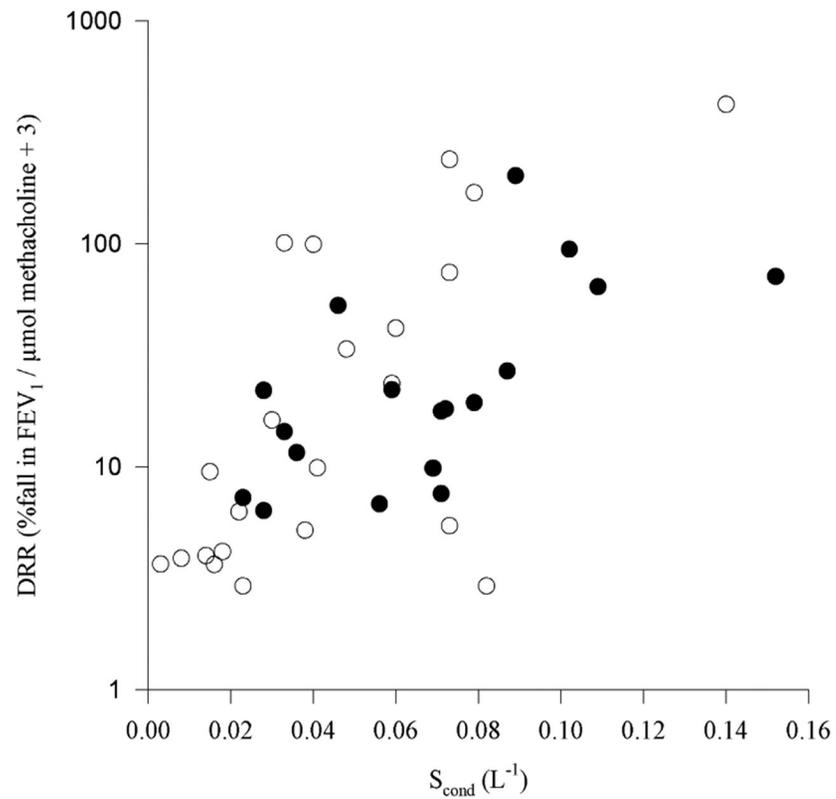
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Figure 1

a)



b)

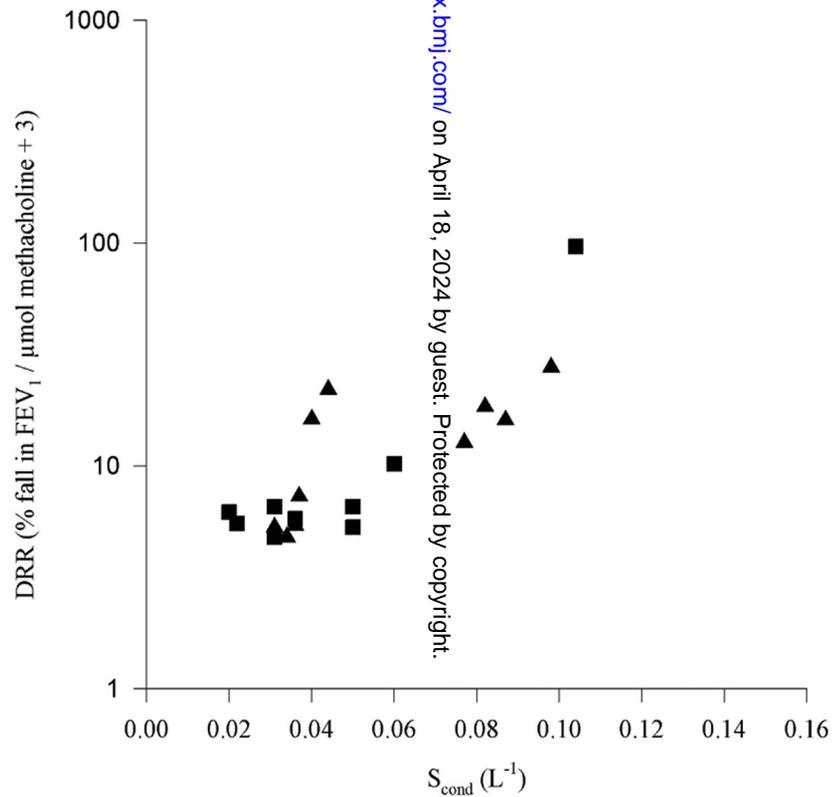


Figure 2

