

Respiratory system reactance is an independent determinant of asthma control

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Kelly VJ, Sands SA, Harris RS, Venegas JG, Brown NJ, Stuart-Andrews CR, King GG, Thompson BR. Respiratory system reactance is an independent determinant of asthma control. *J Appl Physiol* 115: 1360–1369, 2013. First published August 29, 2013; doi:10.1152/jappphysiol.00093.2013.—The mechanisms underlying not well-controlled (NWC) asthma remain poorly understood, but accumulating evidence points to peripheral airway dysfunction as a key contributor. The present study tests whether our recently described respiratory system reactance (Xrs) assessment of peripheral airway dysfunction reveals insight into poor asthma control. The aim of this study was to investigate the contribution of Xrs to asthma control. In 22 subjects with asthma, we measured Xrs (forced oscillation technique), spirometry, lung volumes, and ventilation heterogeneity (inert-gas washout), before and after bronchodilator administration. The relationship between Xrs and lung volume during a deflation maneuver yielded two parameters: the volume at which Xrs abruptly decreased (closing volume) and Xrs at this volume (Xrs_{crit}). Lowered (more negative) Xrs_{crit} reflects reduced apparent lung compliance at high lung volumes due, for example, to heterogeneous airway narrowing and unresolved airway closure or near closure above the critical lung volume. Asthma control was assessed via the 6-point Asthma Control Questionnaire (ACQ6). NWC asthma was defined as ACQ6 > 1.0. In 10 NWC and 12 well-controlled subjects, ACQ6 was strongly associated with postbronchodilator (post-BD) Xrs_{crit} ($R^2 = 0.43$, $P < 0.001$), independent of all measured variables, and was a strong predictor of NWC asthma (receiver operator characteristic area = 0.94, $P < 0.001$). By contrast, Xrs measures at lower lung volumes were not associated with ACQ6. Xrs_{crit} itself was significantly associated with measures of gas trapping and ventilation heterogeneity, thus confirming the link between Xrs and airway closure and heterogeneity. Residual airway dysfunction at high lung volumes assessed via Xrs_{crit} is an independent contributor to asthma control.

airway closure; airway heterogeneity; peripheral airway dysfunction; asthma symptoms; forced oscillation technique; nomenclature

DESPITE OPTIMAL GUIDELINE-defined clinical care, the achievement of asthma control in many patients remains elusive, such that as many as 40% of subjects continue to have not well-controlled (NWC) asthma (3). One of the primary obstacles to the effective management of asthma is an inadequate understanding of the physiological impairment underlying asthma symptoms.

Researchers have intensively investigated the pathophysiological mechanisms responsible for the severity of asthma symptoms. To date, functional assessment of airflow obstruction, ventilation heterogeneity (inert-gas washout), and airway inflammation with exhaled nitric oxide have been found to be modestly associated with the clinical presentation of asthma (asthma control or severity scores, airway hyperresponsiveness, and exacerbation rates) (1, 14–16, 44). Although less commonly assessed, airway mechanical dysfunction in the form of severe heterogeneous peripheral airway narrowing and closure is gaining increased recognition as a characteristic feature of asthma (6, 11, 24, 32). Such dysfunction may be the result of airway inflammation, airway wall remodeling (25), including elevated airway smooth muscle mass (10) and/or local and regional airway-tissue interactions (49). In asthma, peripheral heterogeneous airway narrowing and closure are considered a major cause of ventilation defects revealed with lung imaging (13, 22, 47) and of the alterations in pulmonary oscillatory mechanics (30, 31), specifically lowered respiratory system reactance (Xrs). Since larger and more numerous ventilation defects occur in more severe asthma (2, 13), our central proposal is that the degree of peripheral airway narrowing and closure is an important contributor to asthma control.

We recently developed a technique that utilizes the changes in Xrs with lung volume to concurrently assess closing volume (Vol_{crit}) and Xrs at this closing volume (Xrs_{crit}) (28). Measuring Xrs from total lung capacity (TLC) to residual volume (RV) shows that Xrs rises very slightly [i.e., as lung tissue compliance increases with reduced lung volume (52)], but, when lung volume decreases below a threshold (Vol_{crit}) Xrs decreases precipitously (becomes more negative). This fall in Xrs is consistent with the onset and development of progressive severe airway narrowing and closure. In principle, severe airway narrowing and closure obstructs a proportion of the lung from the pressure oscillations of the forced oscillation technique and, therefore, decreases the apparent lung compliance (compliance = $\Delta\text{volume}/\Delta\text{pressure}$, where Δ is change) seen at the mouth. Vol_{crit} is, therefore, interpreted as a measure of closing volume, much like nitrogen washout; indeed, Vol_{crit} is raised in asthma vs. controls, consistent with the reported increase in closing volume in asthma (35). Xrs_{crit}, which represents the maxima in Xrs with respect to lung volume, is characteristically reduced (more negative) in asthma (28), a finding that demonstrates that reduced apparent compliance occurs even above closing volume in asthma. In asthma, such

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lowered apparent compliance can result from the presence of heterogeneous airway narrowing and closure (8, 47).

Given the marked alterations in $X_{rs,crit}$ and Vol_{crit} in asthma compared with healthy controls, the central aim of the present study was to investigate whether X_{rs} reflects the physiological dysfunction contributing to poor asthma control. We tested the hypothesis that patients with NWC asthma exhibit reduced $X_{rs,crit}$ and increased Vol_{crit} , compared with well-controlled (WC) asthma.

METHODS

Subjects. This study was approved by the Research and Ethics Unit at The Alfred Hospital, Australia. A portion of the data used within the present study has been previously published (28). Twenty-two subjects with asthma (15 men) participated in the study, after providing written, informed consent. All subjects were recruited from the specialist Asthma Clinic at The Alfred Hospital and had doctor-diagnosed asthma according to current guidelines (38). Subjects were current nonsmokers (<10 pack-yr smoking history) and were asymptomatic for acute respiratory infection. All subjects used short-acting bronchodilators as needed and a combined inhaled corticosteroid and long-acting β_2 -agonist. Subjects had invariable lung function over the previous 4 wk (historical lung function measurements reviewed where available); of note, these subjects were previously described (28) as “well-controlled” in this context.

Experimental design. Subjects completed in order: the Asthma Control Questionnaire (ACQ) (27), spirometry, static lung volumes (plethysmography), forced oscillation technique measurements, and multiple-breath nitrogen washout. All tests, excluding ACQ, were completed at baseline and after short-acting β_2 -agonist was administered (300 μ g, salbutamol) via a spacer (Bronchodilator). Before testing, subjects withheld short-acting bronchodilator medications for at least 6 h, and long-acting bronchodilators and inhaled corticosteroid therapies were withheld for at least 12 h.

Equipment and measurements. Spirometry and lung volumes were performed on a Medgraphics Platinum Elite Dx (Medical Graphics, St. Paul, MN), according to American Thoracic Society/European Respiratory Society criteria (37, 51). Spirometric and lung volumes results are reported as percentage predicted, unless otherwise stated. X_{rs} (6 Hz) was assessed using a previously described forced oscillation technique device (43) and analyzed using Matlab (MathWorks, Natick, MA).

Asthma control. Asthma control was assessed using the ACQ (27). The ACQ includes the frequency of night-time awakenings, morning symptoms, limitations to daily activities, shortness of breath, wheeze, short-acting β_2 -agonist use, and forced expiratory volume in 1 s (FEV_1). We used the 6-point ACQ (ACQ6) to exclude the contribution of FEV_1 . Subjects were defined as WC or NWC based on the $ACQ6 \leq 1.0$ (WC) or >1.0 (NWC) (26). NWC asthma encapsulates subjects with poorly controlled asthma ($ACQ > 1.5$) and with partly controlled asthma ($1.0 < ACQ \leq 1.5$) (26).

Reactance-lung volume relationship. X_{rs} was assessed across multiple lung volumes, as previously described (28). Subjects performed, at least in duplicate, a specialized breathing protocol containing three deflation maneuvers (Fig. 1). The breathing protocol consisted of 1 min of tidal breathing, followed by a slow vital capacity maneuver and a further 30 s of tidal breathing. Subsequently, three deflation maneuvers were performed in series, separated by periods of tidal breathing. Finally, another slow vital capacity maneuver was performed at the end of the protocol. Each deflation maneuver consisted of an inspiration to TLC followed by tidal breaths with decreasing end expiratory lung volume until RV was reached. End-inspiration and end-expiration measurements of X_{rs} during each deflation maneuver were collated and plotted against the lung volume at which they were obtained from which two primary measures were determined (Fig.

1B). First, the Vol_{crit} is taken as a surrogate measure of closing volume. An elevation in Vol_{crit} indicates an increased susceptibility for the development of airway closure as lung volume decreases from TLC. Second, $X_{rs,crit}$ is used as a measure of overall apparent lung compliance that reflects the degree of airway heterogeneity and closure or near closure of airways that persists above closing volume (28). Importantly, modeling studies have shown that the degree of airway narrowing required to recreate ventilation defects and/or alterations in apparent lung compliance is largely indistinguishable when airway diameter is reduced to $>70\%$ of baseline (9, 30, 46). It follows that airways with $>70\%$ airway narrowing are functionally closed airways, such that the time constant for ventilation is long relative to breathing frequency or forced oscillation frequency. In the context of our study, we use the term functional airway closure to represent those airways with $>70\%$ airway narrowing.

All subjects were able to satisfactorily perform the X_{rs} measurement. At baseline, the average number of trials performed was 2.5 ± 0.24 (mean $\pm$ SE), and after bronchodilator the average number of trials was 2.3 ± 0.1 . The most common cause for repeating a data protocol was a mouth leak. No significant associations were found between the number of protocols performed and the resulting $X_{rs,crit}$ and Vol_{crit} or ACQ6 at either baseline or after bronchodilator (Pearson correlation).

Multiple-breath nitrogen washout. Multiple-breath nitrogen washout tests were performed as previously described (50) using the exact experimental setup and analysis as described by Stuart-Andrews et al. (45). Briefly, ventilatory flow and the nitrogen concentration at the mouth were continuously recorded during ~ 20 – 30 consecutive 1-liter tidal breaths where 100% oxygen is delivered during inspiration. For each expiration, the phase III slope is determined as the gradient of the relationship between expired volume and nitrogen concentration during the alveolar plateau, which is then normalized by the mean expired nitrogen. Finally, the relationship between normalized phase III slope and lung turnover [cumulative expired volume divided by functional residual capacity (FRC)] is used to determine indexes of ventilation heterogeneity, S_{acin} and S_{cond} . Based on theoretical models (40, 41), S_{acin} is interpreted as an index of ventilation heterogeneity within the acinar lung region resulting from different path lengths for diffusion. Likewise, S_{cond} is an index of ventilation heterogeneity resulting from lung regions with different pressure-volume characteristics (50).

Statistical analysis. Data were analyzed using SigmaPlot (Systat Software, Germany). Two-way repeated-measures ANOVA was used to compare baseline and post-BD results between the WC and the NWC groups. Univariate and forward stepwise regressions were used to assess the association between ACQ6, $X_{rs,crit}$, Vol_{crit} , and additional pulmonary variables (S_{acin} , S_{cond} , FEV_1 , lung volumes), both at baseline and post-BD. Height was included in this analysis as a possible covariate. Receiver operator characteristic analysis was used to statistically test whether $X_{rs,crit}$ and Vol_{crit} are sensitive/specific to the presence of NWC asthma. Data are presented as means $\pm$ SE, unless otherwise stated.

RESULTS

Of the 22 subjects, 10 were defined as NWC and 12 as WC. The WC and NWC groups had similar age, weight, and body mass index; however, the NWC group had a lower height compared with the WC group (Table 1). All subjects were Caucasian. Pulmonary function testing was performed at a similar time of day in the NWC and WC groups. As designed, the NWC group had significantly higher ACQ6 scores compared with the WC group (Table 1). The Global Initiative for Asthma Treatment Step (4), determined based on medication doses, was also significantly higher in the NWC group compared with the WC group. Pulmonary function results, reported as the percentage of predicted (12, 20), are included in Table 1.

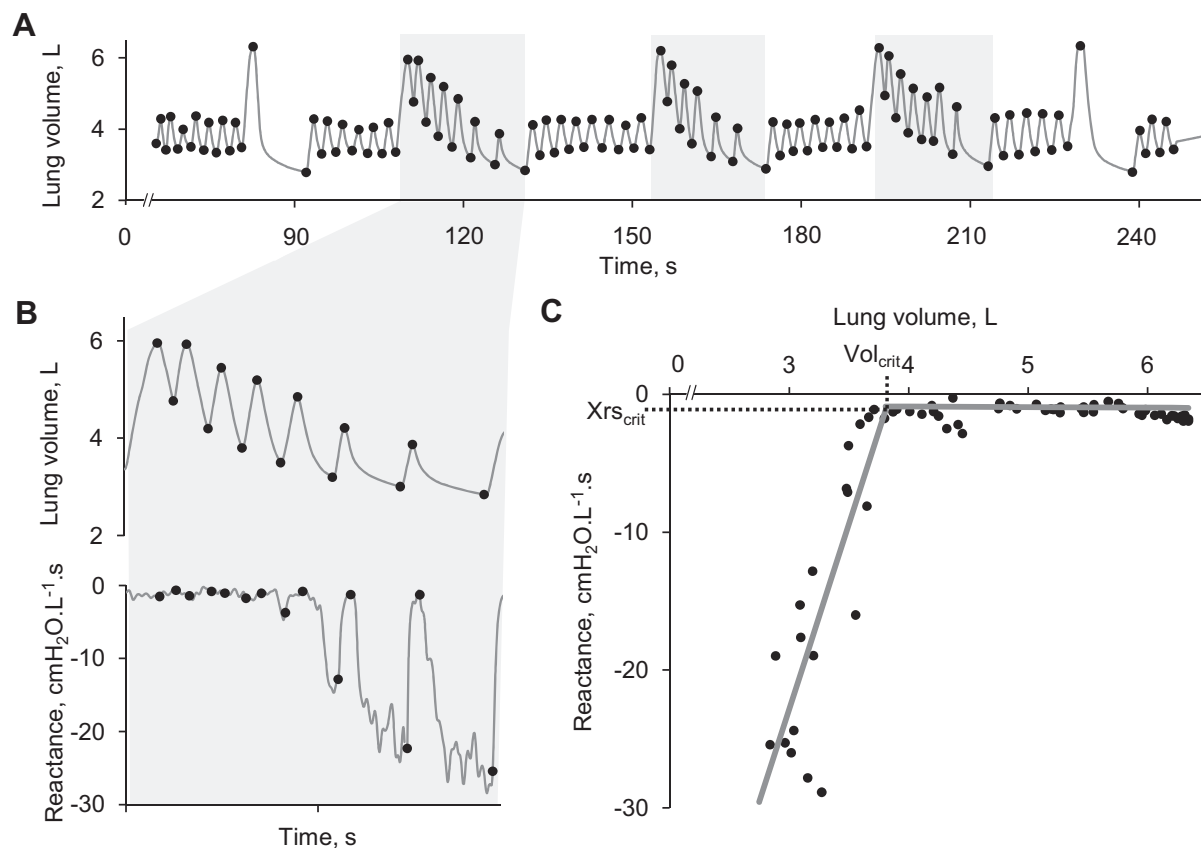


Fig. 1. A: breathing protocol performed to obtain respiratory system reactance (Xrs) vs. lung volume, as recorded from a well-controlled (WC) asthma subject. Shaded regions identify the deflation maneuvers performed that enable Xrs to be calculated between total lung capacity (TLC) and residual volume (RV) at points of zero flow (solid circles at end inspiration and end expiration). Each protocol shown was performed at least in duplicate. B: volume and reactance vs. time during one deflation maneuver, with the zero-flow measurements outlined by the solid circles. C: example Xrs vs. lung volume relationship with bilinear regression. Vol_{crit} is defined as the lung volume where the onset of airway closure occurs. Xrs_{crit} is reactance at this volume, to the right of the precipitous decline with airway closure. Lowered Xrs_{crit} (more negative) reflects a reduced apparent lung compliance (i.e., due to heterogeneous airway narrowing and closure) at high lung volumes (above Vol_{crit}).

The NWC group had a significantly greater degree of airflow obstruction (reduced FEV₁ and FEV₁/forced vital capacity), and a greater degree of gas trapping [indicated by a higher RV and RV/TLC (23)] compared with the WC asthma group.

Reactance and multiple-breath nitrogen washout: NWC vs. WC. The differences between the groups (WC vs. NWC) and conditions (baseline vs. post-BD) for Xrs_{crit}, Vol_{crit}, S_{acin}, and S_{cond}, as determined from two-way repeated-measures ANOVA, are included in Fig. 2; detailed statistics are provided in Table 2. There was a significant effect of group and condition for Xrs_{crit}. Post hoc analysis demonstrated that baseline Xrs_{crit} was significantly lower (more negative) in NWC compared with WC subjects, a difference that became more distinct following bronchodilator. Despite the significant increase in Xrs_{crit} with bronchodilator (main effect, two-way repeated-measures ANOVA), the increase in Xrs_{crit} within WC and NWC groups was not significant (post hoc analysis).

Comparison of post-BD Xrs_{crit} between NWC and WC asthma groups and previously reported healthy controls ($n = 19$) (28) found no difference between WC asthma and healthy controls (-0.83 ± 0.14 vs. -0.62 ± 0.11 cmH₂O.L⁻¹.s). Xrs_{crit} in NWC asthma (-2.02 ± 0.20 cmH₂O.L⁻¹.s) was significantly lower (more negative) than both WC asthma and controls (one-way ANOVA with Student-Newman-Keuls post hoc analysis, both $P < 0.001$).

Baseline Vol_{crit} was significantly higher in NWC compared with the WC group. In both WC and NWC groups, Vol_{crit} decreased following bronchodilator (main effect, $P < 0.001$). Following bronchodilator, Vol_{crit} remained significantly greater in the NWC group compared with the WC group. Post-BD Vol_{crit} increased progressively with disease severity from healthy controls to WC asthma (51.1 ± 2.3 vs. $63.1 \pm 3.8\%$ predicted TLC, $P < 0.01$) to NWC asthma ($78.7 \pm 3.6\%$ predicted TLC, $P < 0.01$ vs. WC and $P < 0.001$ vs. controls; one-way ANOVA with Student-Newman-Keuls post hoc analysis).

S_{acin} was higher in NWC vs. WC, both at baseline and after bronchodilator. Overall, S_{cond} was greater in NWC vs. WC, but only reached significance post-BD. Bronchodilator administration had no effect on S_{acin} or S_{cond}. Overall, there were no differences between WC and NWC groups in terms of the effect size of bronchodilator on Xrs_{crit}, Vol_{crit}, S_{acin}, or S_{cond} (group $\times$ condition).

Factors associated with asthma control. The ACQ6 score was linearly associated with multiple variables, including Xrs_{crit}, Vol_{crit}, S_{acin}, S_{cond}, FEV₁, RV, and RV/TLC (univariate regression; Table 3). Bronchodilator strengthened the associations between ACQ6 and Xrs_{crit}, Vol_{crit}, S_{acin}, and S_{cond}. The strongest association was observed between ACQ6 and Xrs_{crit} post-BD (Fig. 3); Xrs_{crit} explained 43% of the variance in the ACQ6 ($R^2 = 0.43$, $P < 0.001$). Forward stepwise regression

Table 1. Demographics and lung function

	WC Asthma	NWC Asthma		
Demographics				
Age, yr	53.2 ± 3.2	54.0 ± 2.9		
Gender, male/female	9/3	6/4		
Weight, kg	80.0 ± 3.8	82.7 ± 7.3		
Height, cm	174.8 ± 2.9	166.0 ± 2.7*		
BMI, kg/m ²	26.3 ± 1.3	30.2 ± 2.9		
Testing time of day	10:53 ± 39 min	10:32 ± 46 min		
Asthma characteristics				
ACQ6	0.5 ± 0.1	2.0 ± 0.2‡		
GINA treatment step	3.5 ± 0.2	4.1 ± 0.2*§		
	Baseline	Post-BD	Baseline	Post-BD
Spirometry				
FEV ₁ , %predicted	72.5 ± 3.6	76.4 ± 3.2	52.8 ± 5.4†	61.9 ± 5.1*
FVC, %predicted	95.6 ± 4.4	96.8 ± 4.3	82.6 ± 5.4	87.9 ± 3.9
FEV ₁ /FVC, %	58.9 ± 1.7	61.3 ± 1.4	49.1 ± 3.5*	54.2 ± 3.3
Lung volumes				
TLC, %predicted	113 ± 5	111 ± 4	121 ± 3	120 ± 3
FRC, %predicted	107 ± 6	102 ± 6	127 ± 7*	116 ± 7
RV, %predicted	125 ± 5	115 ± 5	162 ± 7‡	147 ± 6‡
RV/TLC, %	39 ± 2	36 ± 2	48 ± 3†	44 ± 2†

Values are means ± SE. WC, well controlled; NWC, not well controlled; BMI, body mass index; ACQ6, 6-point Asthma Control Questionnaire; GINA, Global Initiative for Asthma; BD, bronchodilator; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$: NWC vs. WC asthma. §Comparison performed with $n = 9$ subjects (NWC).

performed using those variables with significant univariate correlations demonstrated that post-BD $X_{rs_{crit}}$ was the only independent determinant of ACQ6. No additional variance in ACQ6 was explained by height, or post-BD Vol_{crit} , S_{acin} , S_{cond} , FEV₁, RV, or RV/TLC.

Receiver operator characteristic analysis (Table 4) identified multiple significant discriminants of NWC and WC asthma, including post-BD values of $X_{rs_{crit}}$, Vol_{crit} , S_{acin} , S_{cond} , and RV, and baseline values of FEV₁ and RV/TLC. Most notably, post-BD $X_{rs_{crit}}$ had a high sensitivity (90%) and specificity

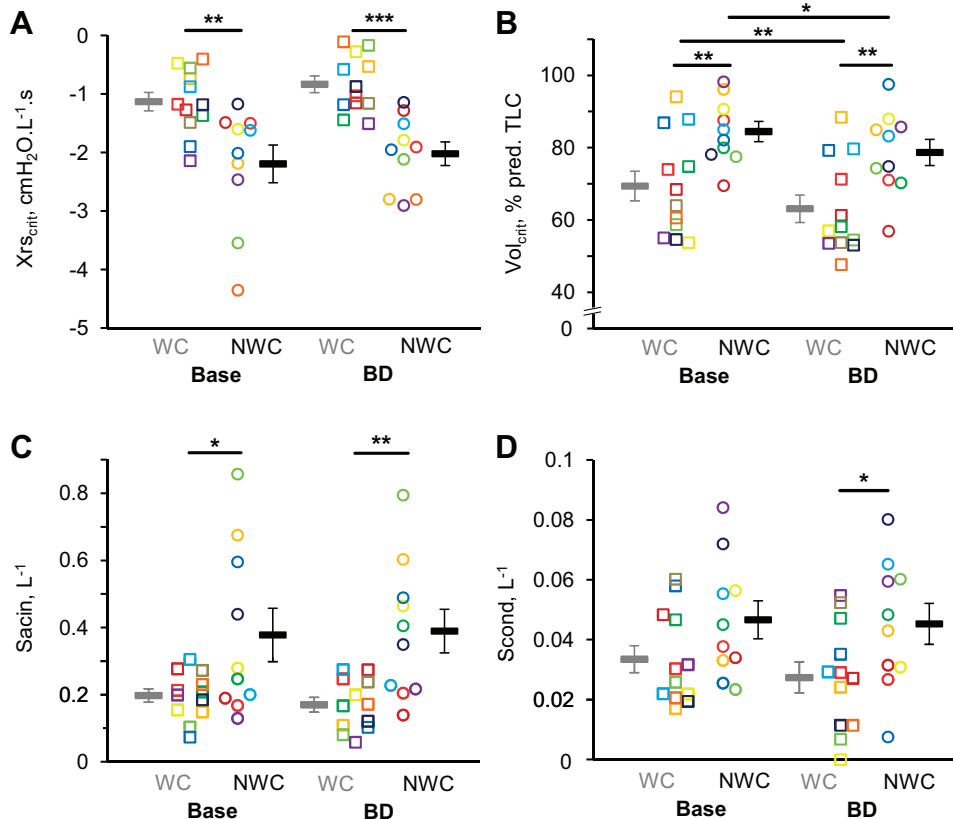


Fig. 2. Reactance and multiple-breath nitrogen washout measures are altered in not well-controlled asthma (NWC; circles, black bars) vs. WC asthma (squares, gray bars). A: $X_{rs_{crit}}$. B: Vol_{crit} . C: ventilation heterogeneity within the acinar lung region (S_{acin}). D: ventilation heterogeneity within the conductive lung region (S_{cond}). Although findings are similar between baseline and post-bronchodilator (BD) conditions, there was a tendency for improved separation of the groups post-BD. Individual subjects within each group are identified with separate colors. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$: two-way repeated-measures ANOVA with Student-Newman-Keuls.

Table 2. Comparison of Xrs_{crit} , Vol_{crit} , S_{acin} , and S_{cond} between WC and NWC groups (two-way repeated-measures ANOVA)

	WC		NWC		Two-Way Repeated-Measures ANOVA, <i>P</i> values		
	Baseline	Post-BD	Baseline	Post-BD	WC vs. NWC group	BD effect	Interaction
Xrs_{crit}	-1.13 ± 0.16	-0.83 ± 0.14	-2.19 ± 0.32^b	-2.02 ± 0.20^c	<0.001	<0.05	
Vol_{crit}	69.4 ± 4.1	63.1 ± 3.8^e	84.5 ± 2.8^b	$78.7 \pm 3.6^{b,d}$	<0.01	<0.001	
S_{acin}	0.20 ± 0.02	0.17 ± 0.02	0.38 ± 0.08^a	0.39 ± 0.06^b	<0.01		
S_{cond}	0.034 ± 0.005	0.027 ± 0.005	0.047 ± 0.006	0.045 ± 0.007^a	<0.05		

Values are means $\pm$ SE. Xrs_{crit} , respiratory system reactance measured at the critical lung volume (Vol_{crit}); S_{acin} and S_{cond} , ventilation heterogeneity within the acinar and conductive lung regions, respectively. ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$: WC vs. NWC post hoc analysis. ^d $P < 0.05$, ^e $P < 0.01$: baseline vs. post-BD post hoc analysis.

(83%) to distinguish between NWC and WC asthma (Fig. 4). Specifically, the threshold of $Xrs_{crit} = -1.23 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$ correctly classified asthma control status in 19/22 subjects.

Factors associated with Xrs_{crit} . To assess whether Xrs_{crit} truly reflects peripheral heterogeneity and airway closure, we examined the factors associated with Xrs_{crit} . Univariate analysis showed that post-BD Xrs_{crit} was significantly associated with measures of ventilation heterogeneity, S_{acin} ($R = -0.57$, $P < 0.01$) and S_{cond} ($R = -0.52$, $P < 0.05$), and indexes of gas trapping and airway closure, RV %predicted ($R = -0.58$, $P < 0.01$) and RV/TLC ($R = -0.75$, $P < 0.0001$). No associations between Xrs_{crit} and FRC or TLC were observed. All regressions were performed using post-BD data.

To confirm the generalizability of associations between Xrs_{crit} and measures of heterogeneity, we repeated the above univariate analysis, including the 19 control subjects from our previous study (28), and found similar results. Xrs_{crit} was significantly associated with S_{acin} ($R = -0.62$, $P < 0.0001$), S_{cond} ($R = -0.43$, $P < 0.01$), RV %predicted ($R = -0.54$, $P < 0.001$), and RV/TLC ($R = -0.61$, $P < 0.0001$). The associations between Xrs_{crit} , S_{acin} , S_{cond} and RV/TLC are included in Fig. 4. Post-BD S_{acin} and S_{cond} in the healthy controls was 0.12 ± 0.02 and $0.018 \pm 0.006 \text{ liter}^{-1}$, respectively.

Factors associated with Vol_{crit} . Using univariate regression, Vol_{crit} was strongly associated with lung volumes, indicating a close connection with available indexes of hyperinflation and gas trapping: RV %predicted ($R = 0.83$, $P < 0.00001$), FRC %predicted ($R = 0.67$, $P < 0.001$), TLC %predicted ($R = 0.78$, $P < 0.00001$), and RV/TLC ($R = 0.62$, $P < 0.01$). There was also an association between Vol_{crit} and S_{acin} ($R = 0.48$, $P < 0.05$) but not S_{cond} . Including controls yielded similar results: RV %predicted ($R = 0.85$, $P < 0.00001$), FRC %predicted ($R = 0.61$, $P < 0.0001$), TLC %predicted ($R = 0.68$, $P < 0.00001$), RV/TLC ($R = 0.77$, $P < 0.01$), S_{acin} ($R = 0.61$,

$P < 0.0001$), and S_{cond} ($R = 0.45$, $P < 0.01$). All regressions were performed using post-BD data.

Lung volume dependence of Xrs and asthma control. To examine whether the strong link between ACQ6 and Xrs relies on the measurement of Xrs at specific lung volumes, post-BD Xrs (determined from the Xrs -lung volume relationship) was measured at RV, FRC, TLC, and a volume midway between FRC and TLC (MID). In general, RV and FRC lie on or near the steep portion of the Xrs vs. lung volume curve. By contrast, MID and TLC lie exclusively on the upper, flatter portion of the Xrs -volume curve. Xrs assessed at each lung volume was compared between WC and NWC (unpaired *t*-test). Xrs measured at RV and at FRC were not significantly different between NWC and WC (Fig. 5). However, Xrs measured at MID and TLC maintained the statistical difference between the NWC and WC group as seen with Xrs_{crit} .

DISCUSSION

Our study shows for the first time that Xrs , measured above closing volume (Xrs_{crit}), is strongly associated with asthma control. Post-BD Xrs_{crit} explains 43% of the variability in asthma symptom scores, independent of all other variables measured in this study. A more negative Xrs reflects a reduced apparent lung compliance; we propose that this reduced apparent compliance in NWC asthma is a consequence of greater peripheral airway closure and heterogeneity (30, 31) vs. WC asthma. This proposal is supported by our finding that the reduced Xrs_{crit} is linked with increased gas trapping (greater RV/TLC) and greater ventilation heterogeneity (S_{cond} , S_{acin}).

Table 3. Univariate correlations (*r* value) between ACQ6 and variables at baseline and post-BD

Variable	Baseline	Post-BD
Xrs_{crit} , $\text{cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$	-0.53^*	$-0.66^\ddagger$
Vol_{crit} , %predicted TLC	0.50^*	$0.59^\ddagger$
S_{acin} , liter^{-1}	0.45^*	$0.55^\ddagger$
S_{cond} , liter^{-1}	0.48^*	0.51^*
FEV ₁ , %predicted	-0.50^*	-0.38^*
FVC, %predicted	-0.26^*	-0.18
RV, %predicted	$0.58^\ddagger$	$0.59^\ddagger$
TLC, %predicted	0.33	0.37
RV/TLC, %	0.49^*	0.47^*
Height, cm	0.49	

* $P < 0.05$, $^\ddagger P < 0.01$, $^\ddagger P < 0.001$.

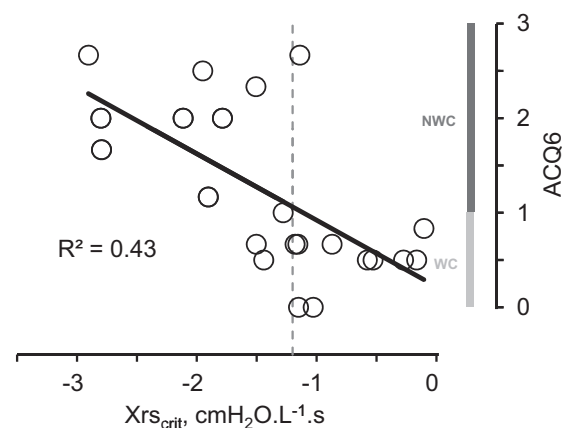


Fig. 3. Association between 6-point Asthma Control Questionnaire score (ACQ6) and post-BD Xrs_{crit} using all subjects, $P < 0.001$. The vertical dashed line indicates the Xrs_{crit} value that best distinguishes between NWC and WC asthma.

Table 4. Receiver operator characteristic analysis

Variable (Optimal Cutoff)		Sensitivity, %	Specificity, %	Area Under Curve	P Value
Xrs _{crit} ($-1.23 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$)	Post-BD	90	83	0.94	<0.001
Vol _{crit} (65.8%predicted TLC)	Post-BD	90	67	0.79	<0.05
S _{acin} (0.20 liter ⁻¹)	Post-BD	90	67	0.84	<0.01
S _{cond} (0.030 liter ⁻¹)	Post-BD	80	67	0.75	<0.05
FEV ₁ (64.6%predicted)	Baseline	80	67	0.78	<0.05
RV (126%predicted)	Post-BD	90	75	0.83	<0.01
RV/TLC (42.6%)	Baseline	70	67	0.81	<0.05

Note: The optimal cutoff point for sensitivity and specificity was determined as the value that maximized both sensitivity and specificity (value closest to the optimal classification).

Taken together, our findings strongly suggest that the presence of peripheral airway heterogeneity and closure is a key identifiable feature of NWC asthma. Moreover, our study demonstrates that this distinguishing dysfunction persists, despite the dilatational forces on the airways provided by the parenchyma at “high” lung volumes (above closing volume), and the dilatational action of inhaled bronchodilator. It follows that the continued presence of elevated peripheral airway heterogeneity and closure at high lung volumes may represent a novel and powerful contributor to asthma control.

Reactance, airway heterogeneity, and closure. On the surface, the lowered (more negative) Xrs in NWC vs. WC asthma might appear to reflect a reduction in the intrinsic tissue compliance of the lung, for example, due to interstitial pulmonary fibrosis (48). However, in general, pulmonary fibrosis is not a phenomenon linked with asthma. Furthermore, lung pressure-volume relationships typically indicate normal lung compliance in asthma (5, 7, 18). Hence, a reduction in the intrinsic lung tissue compliance is an unlikely explanation for the reduced Xrs in NWC vs. WC asthma.

On the other hand, reactance is known to reflect the proportion of closed airways and the degree of peripheral airway heterogeneity by decreasing the apparent lung compliance (29–31) and effectively “hiding” regions of alveolar tissue. Indeed, the associations we report between Xrs_{crit}, Vol_{crit}, and measures of gas trapping (RV, RV/TLC) are consistent with the proposal that Xrs is sensitive to the proportion of closed or severely narrowed airways. RV reflects the portion of lung volume that cannot be passively expired and is influenced by lung elastic recoil and airway closure and narrowing (42). It follows that, in asthma, RV may be elevated due to either an increased susceptibility for airway closure (increased closing volume) or an increased presence of airways that remain functionally closed at all lung volumes (persistent airway closure). Importantly, the presence of functionally closed airways is unlikely to result in regional absorption atelectasis due to the presence of very slow ventilation or collateral ventilation, as has been demonstrated in chronic obstructive pulmonary disease (34).

The relative dominance of Xrs by airway mechanical dysfunction in the peripheral lung is supported by the demonstration that ventilation defects and altered Xrs measured in asthma subjects can be optimally recreated using mathematical modeling by invoking heterogeneous narrowing and closure of peripheral airways, as defined by airway generations 12–16 and below, ~ 0.2 – 2.0 mm diameter (9, 47). This proposal is supported by the associations we observed between Xrs_{crit} and ventilation heterogeneity measured via S_{acin} and S_{cond}. While further validation is required, S_{cond} theoretically reflects the

heterogeneity in specific ventilation due to regional differences in pressure-volume characteristics (compliance), and S_{acin} the asymmetry in lung structure at the acinar level (50). Both of these parameters may be influenced directly by heterogeneities in airway narrowing that results in either different time constants of ventilation (S_{cond}) or an elevation in acinar asymmetry (S_{acin}). In addition, the presence of large contiguous areas of airway closure and near closures (“ventilation defects”) may lead to an exacerbation of the pressure-volume heterogeneities within the lung and therefore act to indirectly elevate S_{cond}. The recently demonstrated relationship between airway closure visualized with SPECT and S_{cond} supports this putative mechanism (17).

In summary, we interpret the reduced (more negative) Xrs_{crit} in NWC vs. WC asthma as a reflection of a greater severity of peripheral airway closure and heterogeneous narrowing at high lung volumes in these patients.

Reactance and asthma control. The observation that Xrs is linked to asthma control when measured above closing volume suggests that the pulmonary deficit that contributes to symptoms is only made visible to the forced oscillation measurement when any additional “volume-dependent” dysfunction below closing volume is minimized. The insensitivity of Xrs at FRC to asthma control suggests that the factor driving poor control is not a reduced Xrs within the tidal breathing range per se; that is, patients may not explicitly sense low apparent compliance at FRC. In fact, we find a poor relationship between Xrs_{crit} and Xrs at FRC ($R^2 = 0.07$, $P = 0.2$), presumably due to the highly variable position of FRC relative to Vol_{crit} (post-BD Vol_{crit} – FRC: WC = 0.23 ± 0.22 , NWC = 0.66 ± 0.18 liters) and the profound effect of lung volume on Xrs below Vol_{crit}. We, therefore, infer that the highly variable peripheral airway dysfunction present at FRC, as evidence by the high slope of the Xrs vs. lung volume relationship near this lung volume reduces the specificity of Xrs at FRC to the important peripheral dysfunction exposed at higher lung volumes that is captured in the Xrs_{crit} measurement.

We found that Xrs_{crit} explains a greater proportion of the variability in asthma control when measured post-BD than when measured at baseline (43 vs. 28%, respectively; Table 2). Likewise, associations between ACQ and Vol_{crit}, S_{acin}, and S_{cond} all strengthened following bronchodilator. This improvement occurred despite a minimal overall effect of bronchodilator on these variables in each group. The cause of the improved associations after bronchodilator may be due to the almost universal reduction in the within-group standard deviation in these measurements after bronchodilator (Table 2). Reduced variability following bronchodilator may be the result

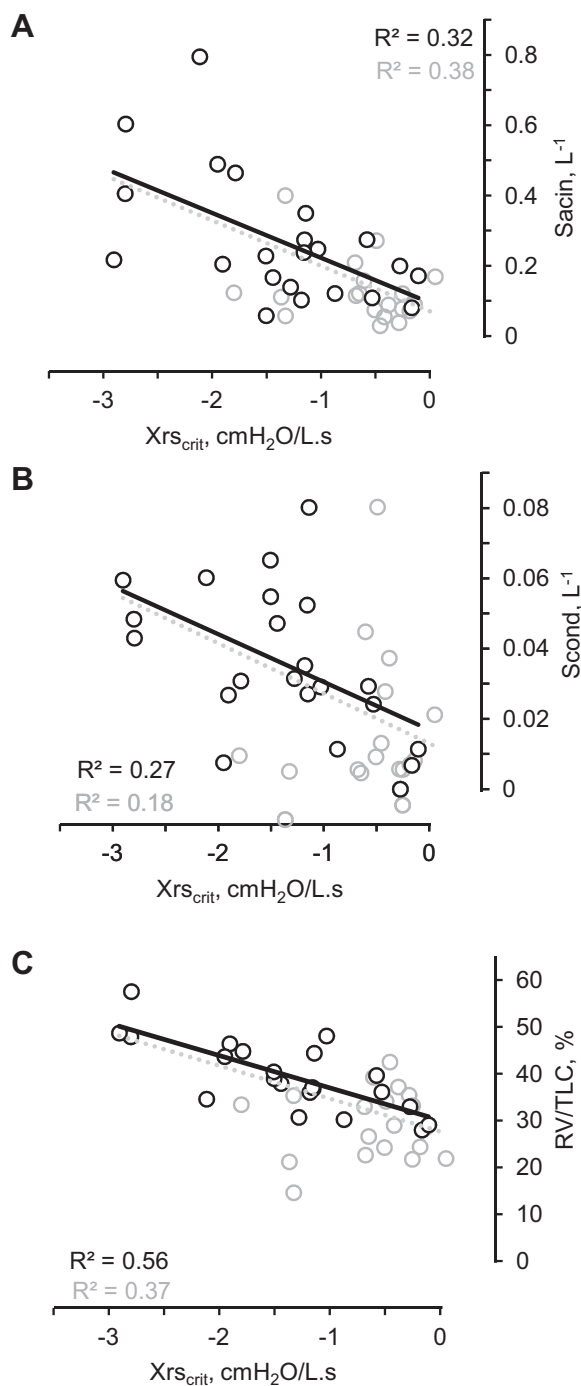


Fig. 4. The individual associations between Xrs_{crit} and measures of ventilation heterogeneity (S_{acin} and S_{cond}) and gas trapping (RV/TLC ratio). Asthma subjects are identified by black circles, and asthma-only regression by solid black line. Control subjects are identified by gray circles, and regression results including asthma and control subject by gray dotted line. A: association between Xrs_{crit} and S_{acin} . B: association between Xrs_{crit} and S_{cond} . C: association between Xrs_{crit} and RV/TLC.

of improved test performance with familiarity of the test procedures; however, the lack of associations between the number of test protocols performed and the resulting Xrs_{crit} and Vol_{crit} at either baseline or after bronchodilator makes this proposal unlikely. Alternatively, it is also possible that some components of the ACQ score may more closely reflect the

symptoms (and physiological deficits) that remain after bronchodilator is used to provide initial symptom relief, such as airway edema, remodeling, or inflammation. Similarly, bronchodilator and lung inflation may serve to remove mild constriction and thereby highlight more fixed pulmonary deficits, which may be responsible for ongoing symptoms.

Our finding that ACQ is associated with peripheral airway dysfunction in the forms of closure and heterogeneity is supported by previous work showing that increased closing volume is a risk factor for asthma exacerbations (24). Further support is provided by the previously demonstrated associations between asthma control and exacerbation rates with ventilation heterogeneity and RV/TLC (6). In addition, a more recent study has demonstrated a modest association between the change in ACQ following inhaled corticosteroid dose titration (up-titration or down-titration) and ventilation heterogeneity, specifically, S_{acin} ($R^2 = 0.13$) and S_{cond} ($R^2 = 0.07$) (15). It follows that there is mounting evidence to support the relevance of peripheral airway heterogeneity and closure in asthma. Our study illuminates the importance of the airway heterogeneity and closure that remains, despite the bronchodilating actions of elevated lung volumes and airway smooth muscle relaxation.

Methodological considerations. A limitation of the present study is the use of a single excitation frequency (6 Hz) for the

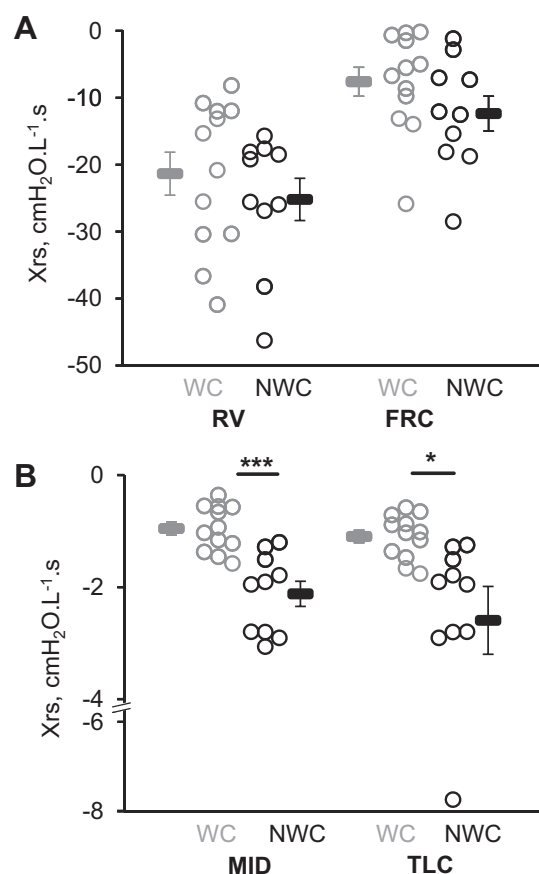


Fig. 5. Lung volume effects on the differences in Xrs between NWC and WC asthma. A: Xrs at RV and functional residual capacity (FRC). B: Xrs at midway between FRC and TLC (MID) and at TLC. Xrs at high lung volumes (MID and TLC) were distinctively lower (more negative) in the NWC vs. WC group, but there were no differences at low lung volumes (RV and FRC). * $P < 0.05$, *** $P < 0.001$: two-way repeated-measures ANOVA with Student-Newman-Keuls.

assessment of Xrs and the inherent inclusion of chest wall compliance in the Xrs measurement. It is well established that Xrs is frequency dependent (21), and, therefore, it is possible that the Xrs-lung volume relationship may have improved sensitivity, if determined across multiple frequencies, as is possible with pseudorandom noise signals (36) or multifrequency sinusoidal signals (33). However, for simplicity, we chose to limit our present investigation to a single frequency. Furthermore, no alterations in chest wall compliance have been reported in asthma, such that our results are unlikely to be explained by a systematically lower chest wall compliance within the NWC asthma group. However, we cannot exclude the possibility that the elevated lung volumes in these subjects have caused some deformation of the chest wall that has lowered its compliance. Furthermore, pleural pressure, at or near TLC, has been reported to be less negative in moderate to severe asthma subjects compared with healthy controls and subjects with mild asthma (7, 18, 19, 53). However, this finding is not universal (5) and remains unexplained. If true, a less negative pleural pressure would result in a reduced airway distending pressure, which may contribute to the preponderance for airway closure at high lung volumes in NWC asthma.

Our study is also inherently limited by the lack of a true gold standard for defining asthma control. An ACQ6 score of <1.0 has been shown to discriminate between NWC and WC asthma when defined by a composite Global Initiative for Asthma/National Institutes of Health gold standard based on asthma symptom diaries and daily peak flow (26); this study reported that this ACQ6 cutoff score has a positive predictive value of 0.83 and a negative predictive value of 0.72 for detecting NWC asthma (26). We feel that major misclassification in the present study is unlikely, given that only 3/22 subjects in the present study had ACQ6 scores in the intermediate range of $0.7 < \text{ACQ6} < 1.6$. Furthermore, an ACQ5 score < 1 (ACQ6 minus rescue inhaler use) was recently shown to be equivalent to a Global Initiative for Asthma definition of WC/partly controlled asthma and a GOAL (Gaining Optimal Asthma Control) definition of totally controlled/WC asthma (39).

We considered the possibility that the lower (more negative) Xrs_{crit} in NWC vs. WC asthma may be driven by the greater hyperinflation (elevated resting lung volumes) present in NWC vs. WC asthma and thus the higher lung volumes at which the Xrs_{crit} measurement is made (note: Xrs falls with increasing volume). However, the change in Xrs across the entire volume range from Vol_{crit} to TLC is minimal (ΔXrs between Vol_{crit} and TLC: WC = -0.26 ± 0.07 ; NWC = -0.57 ± 0.56 cmH₂O·l⁻¹·s) compared with the differences between groups. To illustrate this point, we note that Xrs_{crit} in the NWC subjects is still considerably lower than Xrs at TLC in the WC subjects (NWC Xrs_{crit} = -2.02 ± 0.20 vs. WC Xrs_{TLC} = -1.10 ± 0.11 cmH₂O·l⁻¹·s, $P < 0.001$), demonstrating that differences in lung inflation cannot account for the differences in Xrs_{crit} between NWC and WC groups.

A final potential limitation of the present study was the difference in height between the WC and NWC groups. We accounted for this difference by including it as a covariate within the regression analysis; we found that height was not significantly associated with ACQ6 (univariate regression) and also did not explain any additional variance in ACQ6 that was not explained by Xrs_{crit} (stepwise regression). In addition, we reexamined data from healthy controls (28) to assess whether

height and/or gender might generally explain the intersubject variance in Xrs_{crit} and Vol_{crit}. We found that Xrs_{crit} was not significantly associated with height. However, height did explain 36% of the variance in Vol_{crit} in controls ($R^2 = 0.355$, $P < 0.01$; Vol_{crit} = $-3.575 + 0.0384 \times \text{height}$), a finding that may be expected, given the correlation between lung volumes and height (12). There was also no effect of gender on Xrs_{crit} or Vol_{crit}. Thus it may be important to account for height when assessing the influence of Vol_{crit} on clinical variables, but there is no evidence to suggest that height contributes significantly to Xrs_{crit}.

Clinical implications. Reduced apparent lung compliance that is likely due to airway dysfunction that remains at high lung volumes and following bronchodilator represents a novel contributor to asthma control and a major challenge for the treatment of NWC asthma. Inhaled rescue treatments and/or inhaled controller medications may be particularly ineffective in this group at improving symptoms on the basis that closed/narrowed airways may continue to remain undertreated with inhaled preventative treatments, unless they can be reopened or dilated. Our findings, therefore, highlight the need for novel alternative therapies or techniques that act to dilate persistently narrowed or closed airways to ultimately facilitate appropriate delivery of treatment and, potentially, to aid in the maintenance/achievement of asthma control.

Conclusion. The present study revealed a powerful association between asthma control and the mechanical properties of the lung periphery (Xrs) that is independent of measurements of closing volume, inert-gas washout measures of airway heterogeneity (S_{acin} and S_{cond}), lung volumes, and airflow obstruction (spirometry). Our data provide evidence that strongly suggests that NWC asthma is characterized by the presence of peripheral airway closure and heterogeneous narrowing that persists, despite bronchodilation and the dilational forces applied by the lung parenchyma at high lung volumes. By contrast, Xrs measured at FRC is not related to asthma control. Longitudinal clinical studies are warranted to assess the utility of Xrs_{crit} to guide medical therapy and predict treatment outcomes in patients with asthma.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: V.J.K., N.J.B., G.G.K., and B.R.T. conception and design of research; V.J.K. performed experiments; V.J.K. and C.R.S.-A. analyzed data; V.J.K., S.A.S., R.S.H., J.G.V., N.J.B., G.G.K., and B.R.T. interpreted results of experiments; V.J.K. prepared figures; V.J.K. drafted manuscript; V.J.K., S.A.S., R.S.H., J.G.V., N.J.B., C.R.S.-A., G.G.K., and

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