Short-term variability in respiratory impedance and effect of deep breath in asthmatic and healthy subjects with airway smooth muscle activation and unloading

Alessandro Gobbi,1 Riccardo Pellegrino,2 Carlo Gulotta,3 Andrea Antonelli,2 Pasquale Pomplio,1 Claudia Crimi,4 Roberto Torchio,3 Luca Datto,5 Paolo Parola,6 Raffaele L. Dellaca,1 and Vito Brusasco7

1TBM Lab, Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano, Milano, Italy; 2Allergologia e Fisiopatologia Respiratoria, ASO S. Croce e Carle, Cuneo, Italy; 3Pneumologia-Fisiopatologia Respiratoria, AOU S. Luigi Gonzaga, Orbassano (Torino), Italy; 4Sezione di Malattie dell’Apparato Respiratorio, Università di Catania, Catania, Italy; 5Medicina d’Urgenza, ASO S. Croce e Carle, Cuneo, Italy; 6Anestesia, ASO S. Croce e Carle, Cuneo, Italy; and 7Fisiopatologia Respiratoria, Dipartimento di Medicina Interna, Università di Genova, Genova, Italy

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Gobbi A, Pellegrino R, Gulotta C, Antonelli A, Pomplio P, Crimi C, Torchio R, Datto L, Parola P, Dellaca RL, Brusasco V. Short-term variability in respiratory impedance and effect of deep breath in asthmatic and healthy subjects with airway smooth muscle activation and unloading. J Appl Physiol 115: 708–715, 2013. First published June 13, 2013; doi:10.1152/japplphysiol.00013.2013.—Inspiratory resistance (RINSP) and reactance (XINSP) were measured for 7 min at 5 Hz in 10 subjects with mild asymptomatic asthma and 9 healthy subjects to assess the effects of airway smooth muscle (ASM) activation by methacholine (MCh) and unloading by chest wall strapping (CWS) on the variability of lung function and the effects of deep inspiration (DI). Subjects were studied at control conditions, after MCh, with CWS, and after MCh with CWS. In all experimental conditions XINSP was significantly more negative in subjects with asthma than in healthy subjects, suggesting greater inhomogeneity in the former. However, the variability in both RINSP and XINSP was increased by either ASM activation or CWS, without significant difference between groups. DI significantly reversed MCh-induced changes in RINSP both in subjects with asthma and healthy subjects, but XINSP in the former only. This effect was impaired by CWS more in subjects with asthma than in healthy subjects. The velocity of RINSP and XINSP recovery after DI was faster in subjects with asthma than in healthy subjects. The velocity of RINSP activation and unloading.

In asthmatic and healthy subjects with airway smooth muscle activation and unloading, the relationship between the increased variability in lung function (29, 37, 46) and also in healthy subjects after ASM activation by methacholine (MCh) or shifting from upright to supine position (23). Altogether, the above findings would suggest that the variability in lung function may reflect intrinsic properties of ASM or unloading, independent of disease. Yet the relationships between the increased variability in lung function (29, 37, 46), the reduced bronchodilator effect of DI (10), and the increased velocity of airway narrowing observed in bronchial asthma remain elusive.

Imaging studies have shown that ventilation defects develop during acute bronchoconstriction in asthma (5, 36, 48, 56–58), which can be attributed to heterogeneous distribution of airway remodeling (26) and impedance (53). Respiratory impedance is a complex parameter with two dimensions; namely, resistance (Rs) and reactance (Xr), the former mainly reflecting the properties of conducting airways and the latter the capacitive properties of peripheral airways and lung parenchyma (19, 21, 50). Because small airways have been shown to be involved in the remodeling process of asthma, it can be hypothesized that ASM activation, unloading, and deep inspiration cause greater changes in Xr than in people with asthma but not in healthy subjects. Alternatively, Xr and Rs should change similarly to an increase in airway smooth muscle (ASM) shortening. Whether this represents an alteration of ASM phenotype in asthma has been the object of recent debate (31, 42).

Studies in vitro comparing the mechanical properties of ASM in subjects with and without asthma have provided conflicting results, with some showing differences (2, 4, 6, 31, 33, 55, 61) and others no differences (12, 13, 14, 60) in contractile properties. In vivo, the picture is more complicated by several mechanisms that may modulate ASM shortening and airway narrowing. Because ASM shortening is the result of the balance between the force ASM generates and the loads it must act against (10, 39), any reduction in load will result not only in a shorter ASM for any given tone (2, 25, 49) but also in an increased velocity of shortening (25, 39, 52). Additional consequences of ASM unloading are that ASM may adapt to operate at reduced length, where it can shorten more (8, 30, 49), and its ability to relax during tidal or deep inspiration (DI) is reduced (1). In addition, an increased velocity in airway narrowing has been found to correlate with an increased within-breath variability in respiratory resistance (47). Both short- and long-term variabilities in respiratory impedance were found to be increased in subjects with asthma (29, 37, 46) and also in healthy subjects after ASM activation by methacholine (MCh) or shifting from upright to supine position (23)

BRONCHIAL ASTHMA IS FUNCTIONALLY characterized by exaggerated and variable airway narrowing in response to a variety of stimuli. These abnormalities are believed to be the result of airway inflammation and remodeling (9), but the presence of inflammatory cells in the airways is neither necessary (15) nor sufficient (16) to explain airway hyperresponsiveness. These observations and the similarity of bronchoconstrictor responses to widely different stimuli suggest that a key mechanism for exaggerated and variable airway narrowing in asthma is an abnormal response of the target organ, mainly due to an increase in airway smooth muscle (ASM) shortening. Whether this represents an alteration of ASM phenotype in asthma has been the object of recent debate (31, 42).

Studies in vitro comparing the mechanical properties of ASM in subjects with and without asthma have provided conflicting results, with some showing differences (2, 4, 6, 31, 33, 55, 61) and others no differences (12, 13, 14, 60) in contractile properties. In vivo, the picture is more complicated by several mechanisms that may modulate ASM shortening and airway narrowing. Because ASM shortening is the result of the balance between the force ASM generates and the loads it must act against (10, 39), any reduction in load will result not only in a shorter ASM for any given tone (2, 25, 49) but also in an increased velocity of shortening (25, 39, 52). Additional consequences of ASM unloading are that ASM may adapt to operate at reduced length, where it can shorten more (8, 30, 49), and its ability to relax during tidal or deep inspiration (DI) is reduced (1). In addition, an increased velocity in airway narrowing has been found to correlate with an increased within-breath variability in respiratory resistance (47). Both short- and long-term variabilities in respiratory impedance were found to be increased in subjects with asthma (29, 37, 46) and also in healthy subjects after ASM activation by methacholine (MCh) or shifting from upright to supine position (23). Altogether, the above findings would suggest that the variability in lung function may reflect intrinsic properties of ASM or unloading, independent of disease. Yet the relationships between the increased variability in lung function (29, 37, 46), the reduced bronchodilator effect of DI (10), and the increased velocity of airway narrowing observed in bronchial asthma remain elusive.

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in subjects with asthma and those without asthma if they reflect intrinsic properties of ASM. To test these hypotheses, we measured Rrs and Xrs in healthy men and those with mild asymptomatic asthma to assess the effects of ASM activation by MCh and unloading by chest wall strapping (CWS) on 1) the temporal and spatial variability of lung function, 2) the bronchodilator effect of DI, and 3) the velocity of airway narrowing after DI.

METHODS

Subjects

Ten men with mild, intermittent bronchial asthma (27) and 9 age-matched healthy men were studied (Table 1). To be included, subjects with asthma had to be in stable clinical condition in the previous 4 wk without regular asthma treatment. The study protocol was approved by the local Ethical Committee and written informed consent was obtained from each subject before entering the study.

Lung Function Measurements

Spirometry, maximal flow-volume curves, and absolute lung volumes were obtained with a body plethysmograph (Autobox; SensorMedics, California) following American Thoracic Society/European Respiratory Society recommendations (41, 59). Briefly, after at least four regular breaths, thoracic gas volume (TGV) was measured while subjects were panting against a closed shutter at a frequency slightly <1 Hz with cheeks supported by hands. After the shutter was opened, the subject took a full inspiratory capacity (IC) and then forcefully expired from total lung capacity (TLC) to residual volume (RV) for at least 4 s to measure forced vital capacity (FVC) and 1-s forced expiratory volume (FEV1). Functional residual capacity (FRC) was calculated from TGV, corrected for any difference between the volume at which the shutter was closed and the average end-expiratory tidal volume of previous tidal breaths. Predicted values for spirometry and lung volumes were obtained from Quanjer et al. (45).

Respiratory impedance was measured by a forced oscillation system previously described (20, 28, 29). Sinusoidal pressure oscillations (5 Hz frequency, ~2 cmH2O amplitude) were generated by a 16-cm-diameter loudspeaker (CW161N; Ciare, Italy) and applied at the mouth while subjects were breathing normally at a frequency of ~20 breaths/min set by a metronome and supporting their cheeks by hands. The loudspeaker was mounted in a rigid box and connected in parallel to a mesh pneumotachograph and mouthpiece on one side, and to a low-resistance, high-inertance tube on the other (overall load at the frequency of normal breathing = 0.98 cmH2O·s·L⁻¹). Airway opening pressure and flow were recorded by piezoresistive differential transducers (DCXL10DS and DCXL01DS, respectively; Sensortechnics, Germany) and sampled at 200 Hz. A 15 L/min bias flow of air generated by an air pump (CMP08; 3A Health Care, Italy) was used to reduce the system dead space to ~35 mL. Respiratory resistance and reactance (Rrs and Xrs, respectively) were measured over the whole breath, and separately for inspiration (RINS and XINS, respectively) and expiration (REXP and XEXP, respectively) by a least squares algorithm (34, 35). Only RINS and XINS were used for the present analysis. Artifacts due to glottis closure or expiratory airflow limitation were avoided by automatically discarding breaths showing any of the following features: 1) tidal volume <0.1 L or >2.0 L; 2) difference between measured flow oscillation and ideal sine wave with the same Fourier coefficients >0.2 (40); and 3) ratio of minimum to average Xrs >3.5 (28). Visual inspection of each test was performed to evaluate the overall quality of Rrs and Xrs.

Study Protocol

On a prestudy day after spirometry and lung volume measurements, subjects underwent an incremental-dose inhalation challenge to determine the single dose of MCh to be used in the subsequent study days. Doubling MCh doses from 20 to 1,200 μg for subjects with asthma and from 300 to 4,800 μg for healthy subjects, were inhaled until FEV1 was decreased by 20% or more from baseline, or the next dose should have been >5,000 μg. For this purpose, dry-powder MCh chloride (Laboratorio Farmaceutico Lofarma, Italy) dissolved in 3 ml of distilled water was aerosolized by an ampoule-dosimeter system (MB3; MEFAR, Italy) delivering particles with a median mass diameter ranging between 1.53 and 1.61 μm, and inhaled during spontaneous quiet breathing in a sitting position. The dose causing a 20% decrease in FEV1 (PD20FEV1) was calculated by interpolation of the dose-response curve.

Subjects then attended the laboratory on two other occasions at least 1 wk apart to undergo single-dose MCh challenges using the last dose achieved on the prestudy day, and with or without CWS in a random order. On the first study day, after control spirometry and lung volume measurements, respiratory impedance was determined over 7 min of tidal breathing with a DI taken at the end of the fifth minute. All measurements were taken again after a single dose of MCh. On the second study day, a corset constraining the whole chest wall was applied with the help of two elastic and two inelastic corsets extending from axillae to lower abdomen. All measurements were taken before and after MCh as on the first study day. All subjects were instructed to avoid unrequested larger than regular breaths or sighs during the studies. At the end of each study day, subjects were given aerosol albuterol for symptoms relief.

Data Analysis

The variability in RINS and XINS was estimated from the interquartile range (IQR) of their respective probability density estimations (RINS_IQR and XINS_IQR) using all regular breaths before DI. A linear regression analysis was applied to all values of RINS and XINS measured from the end of DI to the point at which a clear plateau was observed (Fig. 1). The intercepts of regression lines at the time DI was terminated (RINS_Plate and XINS_Plate) were compared with the average pre-DI values to estimate the bronchodilator effects of DI, and slopes (RINS_Slope and XINS_Slope) were taken as estimates of renarrowing rates (44). An unpaired t-test was used to evaluate the statistical significance of differences in anthropometric and baseline functional characteristics between groups. A within-between groups repeated measures ANOVA with the Holm-Sidak multiple comparison test was used to assess differences between conditions and groups. Relationships between variables were tested by simple regression analysis. P < 0.05
RESULTS

There were no significant differences in anthropometric characteristics, spirometry, or lung volumes between subjects with asthma and healthy subjects (Table 1). Presumably, this was because subjects had a very mild form of bronchial asthma and were free from functional defects at the time of the study. \( \text{PD}_{20}\text{FEV}_1 \) was expectedly less \((P < 0.001)\) and \( X_{\text{INSP}} \) was more negative \((P = 0.021)\) in subjects with asthma under all experimental conditions (Table 2).

**Effects of MCh**

Inhalation of MCh caused variable decrements in \( \text{FEV}_1 \) \((P < 0.001)\) and \( \text{FVC} \) \((P = 0.007)\), and increments in \( \text{FRC} \) \((P < 0.001)\) and \( \text{RV} \) \((P < 0.001)\) that were not significantly different between subjects with asthma and healthy subjects (Table 2). \( R_{\text{INSP}} \) increased significantly from control conditions \((P < 0.001)\) and \( X_{\text{INSP}} \) became more negative \((P = 0.001)\), but without significant difference between groups. Also, \( R_{\text{INSP-IQR}} \) and \( X_{\text{INSP-IQR}} \) increased significantly \((P = 0.006 \text{ and } P = 0.002, \text{ respectively})\) without significant difference between groups (Fig. 2). DI reduced \( R_{\text{INSP}} \) significantly \((P < 0.001)\) in both healthy subjects and those with asthma and made \( X_{\text{INSP}} \) less negative in those with asthma \((P = 0.001)\) and healthy subjects \((P = 0.107)\), reaching values that were not significantly different from control \((P = 0.133 \text{ and } P = 0.914, \text{ respectively})\), and without significant difference between groups (Fig. 3). \( R_{\text{INSP-Slope}} \) and \( X_{\text{INSP-Slope}} \) increased significantly in subjects with asthma \((P = 0.001 \text{ for both})\) but not in healthy subjects \((P = 0.133 \text{ and } P = 0.708, \text{ respectively})\) (Fig. 4).

**Effects of CWS**

Expectedly, CWS caused significant reductions in TLC \((P < 0.001)\), FRC \((P < 0.001)\), \( \text{FVC} \) \((P < 0.001)\), and \( \text{FEV}_1 \) \((P < 0.001)\), but not \( \text{RV} \) \((P = 0.617)\), without significant difference between healthy subjects and those with asthma (Table 2). \( R_{\text{INSP}} \) increased significantly from control \((P = 0.009)\) but less than with MCh \((P = 0.001)\) without significant difference between groups. \( X_{\text{INSP}} \) was more negative than at control \((P < 0.001)\), but not significantly different from MCh \((P = 0.105)\). \( R_{\text{INSP-IQR}} \) and \( X_{\text{INSP-IQR}} \) were significantly increased \((P = 0.003 \text{ and } P < 0.001, \text{ respectively})\) to values that were similar to those with MCh \((P = 0.842 \text{ and } P = 0.707, \text{ respectively})\), without significant difference between groups (Fig. 2). DI had no significant effects on \( R_{\text{INSP}} \) and \( X_{\text{INSP}} \) (Fig. 3); \( R_{\text{INSP-Slope}} \) and \( X_{\text{INSP-Slope}} \) were not significantly different from control (Fig. 4).
Changes in lung function with methacholine, chest wall strapping, or both

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<tr>
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<th>Control</th>
<th>MCh</th>
<th>CWS</th>
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<tr>
<td>FEV₁, L</td>
<td>3.74 ± 0.77</td>
<td>3.07 ± 0.70</td>
<td>2.38 ± 0.55</td>
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<td>FVC, L</td>
<td>4.95 ± 1.03</td>
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<td>TLC, L</td>
<td>6.66 ± 1.30</td>
<td>6.66 ± 1.31</td>
<td>5.03 ± 0.91</td>
<td>5.13 ± 1.07</td>
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<td>FRC, L</td>
<td>3.00 ± 0.91</td>
<td>3.45 ± 0.99</td>
<td>2.54 ± 0.66</td>
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<td>RV, L</td>
<td>1.76 ± 0.58</td>
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<td>RINSp, cmH₂O·s·L⁻¹</td>
<td>3.00 ± 1.11</td>
<td>4.97 ± 1.83</td>
<td>3.86 ± 1.43</td>
<td>5.44 ± 1.37</td>
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<td>XINSp, cmH₂O·s·L⁻¹</td>
<td>−0.93 ± 0.50</td>
<td>−2.51 ± 2.01</td>
<td>−2.96 ± 1.35</td>
<td>−4.52 ± 1.82</td>
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<th>Healthy Subjects</th>
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<td>FEV₁, L</td>
<td>4.02 ± 0.46</td>
<td>3.55 ± 0.50</td>
<td>2.86 ± 0.55</td>
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<td>FVC, L</td>
<td>4.98 ± 0.63</td>
<td>4.69 ± 0.70</td>
<td>3.58 ± 0.62</td>
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<td>TLC, L</td>
<td>6.42 ± 0.87</td>
<td>6.54 ± 0.87</td>
<td>5.01 ± 0.62</td>
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<td>FRC, L</td>
<td>2.98 ± 0.45</td>
<td>3.28 ± 0.53</td>
<td>2.22 ± 0.38</td>
<td>2.85 ± 0.74</td>
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<td>RV, L</td>
<td>1.35 ± 0.50</td>
<td>1.80 ± 0.49</td>
<td>1.45 ± 0.54</td>
<td>2.04 ± 0.92</td>
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<td>RINSp, cmH₂O·s·L⁻¹</td>
<td>2.25 ± 0.28</td>
<td>3.96 ± 1.52</td>
<td>3.02 ± 1.24</td>
<td>4.59 ± 1.13</td>
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<td>XINSp, cmH₂O·s·L⁻¹</td>
<td>−0.62 ± 0.21</td>
<td>−1.06 ± 0.66</td>
<td>−1.57 ± 0.87</td>
<td>−3.18 ± 0.96</td>
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MCh, methacholine; CWS, chest wall strapping; RINsp, inspiratory resistance at 5 Hz; XINsp, inspiratory reactance at 5 Hz. See Table 1 for other abbreviations. Pairs of symbols indicate statistically significant differences between conditions as estimated by Holm-Sidak multiple comparison test. Data are means ± SD.

Effects of MCh in the Presence of CWS

Inhalation of MCh caused greater decrements in FEV₁ (P < 0.001) and FVC (P < 0.001), and increments in FRC (P < 0.001) and RV (P = 0.026) than in the absence of CWS, without significant differences between groups (Table 2).

RINsp tended to increase (P = 0.070) and XINsp became significantly more negative (P < 0.001) than in the absence of CWS, without significant difference between groups. Both RINsp_IQR and XINsp_IQR further increased compared with MCh alone (P = 0.004 and P < 0.001, respectively), without significant difference between groups (Fig. 2). The attenuating effects of DI on RINsp were maintained only in healthy subjects (P = 0.048), though at a significantly lower level than with MCh alone (P < 0.001 for both). Neither RINsp_IQR nor XINsp_IQR were significantly different from control (Fig. 4).

Relationships Between Variables

RINsp_IQR was significantly correlated with RINsp in all conditions and in both groups, except in the healthy subjects at control conditions, with similar regression coefficients that tended to increase with MCh plus CWS (Table 3), whereas XINsp_IQR correlated with XINsp in subjects with asthma only. With MCh alone, RINsp_IQR was significantly correlated with RINsp_IQR in both subjects with asthma (r² = 0.50) and healthy subjects (r² = 0.69), whereas XINsp_IQR correlated with XINsp_IQR only in the former (r² = 0.88). In addition, in subjects with asthma but not in healthy subjects, RINsp_IQR was negatively correlated with XINsp_IQR (r² = 0.85, P < 0.001).

DISCUSSION

The main results of the present study are as follows: 1) in subjects with mild asthma and standard lung function tests within normal ranges, XINsp was significantly more negative than in healthy subjects; 2) the variability in both RINsp and XINsp was increased by either ASM activation or CWS without significant differences between healthy subjects and those with asthma; 3) DI significantly reversed MCh-induced changes in RINsp in both healthy subjects and those with asthma, and it also reduced XINsp in subjects with asthma; 4) the effect of DI was impaired by CWS more in subjects with asthma than healthy subjects; and 5) reconstitution after DI, as estimated from RINsp_IQR and XINsp_IQR, was faster in subjects with asthma than healthy subjects.
Comments on Methodology

This study has strengths and limitations. Methodological strengths include the following: 1) at variance with previous studies, Rrs and Xrs were computed over the inspiratory portion of tidal breathing only (RINS P and XINS P, respectively), which avoids the artifacts of expiratory flow limitation (17–19); 2) impedance measurements were taken at a fixed breathing frequency of 10–20 breaths/min, which preliminarily has been shown to provide a probability density estimation of RINS P that is substantially narrower than at variable breathing frequency; and 3) the variability in RINS P and XINS P was estimated from IQR rather than coefficient of variation to minimize the effects of skewed probability density estimation, especially under conditions of severe airflow obstruction. Limitations include the following: 1) CWS was used to unload ASM by decreasing the operative lung volume and to limit the amplitude of strain during DI, although it is known to increase lung elastic recoil (51, 54), thus increasing the stress on ASM particularly at full lung inflation; 2) only individuals with mild asthma were studied to minimize the confounding effects of airway inflammation, which makes our results not generalizable to the entire population of people with asthma; and 3) a single frequency of 5 Hz was used to assess lung mechanics in asthma, as in previous studies (17, 18, 20, 23, 28, 29, 38, 46), but it is possible that multiple higher frequencies may provide more information on ventilation heterogeneities (38, 50).

Interpretation of Results

Although PD20FEV1 was expectedly lower in subjects with asthma than healthy subjects, no differences in standard lung

Fig. 3. Inspiratory resistance (RINS P) and reactance (XINS P) before and after a DI at control conditions, after MCh, with CWS, and after CWS + MCh in 10 subjects with asthma (A and C) and 9 healthy subjects (B and D). RINS P and XINS P after the DI were estimated from the intercept of the linear regression of values recorded from the end of DI to the time point at which a plateau was reached. Error bars indicate SD. Statistical differences between pre- and post-DI values as estimated by ANOVA with a post hoc multiple comparison test are shown. See text for complete statistical analysis.

Fig. 4. Slope of the linear regression of RINS P (□ and ●) and XINS P (■ and ▼) from the end of deep inspiration to the time point at which a plateau was reached in 10 subjects with asthma (closed symbols) and 9 healthy subjects (open symbols) at control conditions, after MCh, with CWS, and after CWS + MCh. Error bars indicate SD. Statistical differences between subjects with asthma and healthy subjects as estimated by ANOVA with a post hoc multiple comparison test are shown for RINS P (*P < 0.02) and XINS P (#P < 0.002).
function data were observed between the groups in response to the single dose of MCh corresponding to the last cumulative dose given on the prestudy day. Therefore, it seems reasonable to interpret the results of the present study in the frame of ASM dynamics with minimal confounding effects.

Variability in respiratory impedance. In all experimental conditions, $X_{\text{INSPP}}$ was significantly more negative in subjects with asthma than healthy subjects. Because the two groups had very close FRC under any condition, this finding suggests an increased mechanical inhomogeneity ($50$). However, the similarity in $X_{\text{INSPP}}$ between groups suggests that the temporal variability of spatial inhomogeneity was not increased in these subjects with mild asthma.

In accord with a recent study ($44$), ASM activation by MCh caused an increase in $R_{\text{INSP}}$ that was associated with a more negative $X_{\text{INSP}}$, which is consistent with several studies showing that induced bronchoconstriction is associated with increased ventilation inhomogeneities ($5, 11, 26, 36, 50, 56 – 58$). Imaging studies in animals and humans have documented that induced bronchoconstriction is associated with large and patchy ventilation defects, occurring presumably as a result of heterogeneous closure or near-closure of small airways ($5, 11, 34, 48, 58 – 60$). Model simulations showed that small, random perturbations in airway wall thickness upon ASM activation are sufficient to activate a cascade of events leading to ventilation defects ($3$).

With CWS, $R_{\text{INSP}}$ increased in both groups, but this increase was entirely accounted by the reduction in lung volume (constant $R_{\text{INSP}} \times \text{FRC}$). By contrast, $X_{\text{INSP}}$ became more negative than expected from the decrease in FRC, suggesting additional heterogeneity with CWS.

Both $R_{\text{INSP}}$ and $X_{\text{INSP}}$ increased after either MCh or CWS without significant differences between subjects with asthma and healthy subjects, indicating similar temporal and spatial fluctuations in lung function. Altogether, these findings can be interpreted as suggesting that ventilation inhomogeneity and temporal fluctuations in respiratory impedance are typical features of ASM operating at short length independent of contractile status or disease ($36, 56$).

Under all conditions and in both groups, $R_{\text{INSP}}$ was significantly correlated with $R_{\text{INSP}}$, with regression slopes that, with the exception of control, were similar with MCh or CWS.

These findings confirm the results of previous studies in healthy subjects and those with asthma ($23, 46$) and support the notion that the relationship may originate from the inverse dependence of $R_{\text{INSP}}$ on the fourth-power of the airway diameter ($22$). However, the regression slope after MCh with CWS tended to increase, thus suggesting that additional factors other than reduced ASM length contributed to increased $R_{\text{INSP}}$. Were the short length of ASM the only determinant of the relationship between variability and mean $R_{\text{INSP}}$, then the regression slopes should have remained unchanged, which was not the case. In the absence of a clear explanation, we speculate that the increased variability in response to MCh with CWS in asthma is the result of the heterogeneous effects of the decrease in FRC on ventilation, as also suggested by the Del Leary et al. model when airway diameter variability is increased at a fixed mean ($22$).

Effects of deep inspiration. In both groups, deep inspiration was able to fully reverse MCh-induced changes in $R_{\text{INSP}}$ and $X_{\text{INSP}}$, suggesting that the increased ASM tone did not impair airway distensibility. The only significant difference between subjects with asthma and healthy subjects was a faster recovery rate for both $R_{\text{INSP}}$ and $X_{\text{INSP}}$ after the DI, thus suggesting increased velocity of airway renarrowing and closure with time, respectively, as previously reported ($7, 47$). This was associated with a reduced bronchodilator effect of DI. In theory, both airway renarrowing and effect of DI should depend on load and airway tone, and ASM shortening velocity. In asthma, load may be decreased because of loss of interdependence or lung elastic recoil ($10, 39$), and airway tone may be increased because of an increased ASM mass or force generation capacity ($2, 4, 6, 32, 33, 55, 61$). The increased velocity of renarrowing despite a similar bronchodilator effect of DI in both subjects with asthma and healthy subjects is consistent with a greater velocity of ASM shortening in the former ($24, 52$), without differences in ASM tone and load. The variance with the previous study might be explained by a different ASM contractile force, or a reduced load, or both in subjects with more severe asthma. In support of this hypothesis is the observation that when the airway strain was reduced by CWS, then the bronchodilator effect of DI was almost abolished in both groups, but it was significantly less in healthy subjects than in those with asthma. Because CWS is associated

### Table 3. Regression analysis between variability and mean values of respiratory impedance

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<th></th>
<th>Control</th>
<th>MCh</th>
<th>CWS</th>
<th>CWS + MCh</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{INSP}}$ vs. $R_{\text{INSP}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.58</td>
<td>0.50</td>
<td>0.45</td>
<td>0.44</td>
</tr>
<tr>
<td>Slope</td>
<td>0.13 (0.04, 0.21)</td>
<td>0.12 (0.03, 0.21)</td>
<td>0.14 (0.01, 0.26)</td>
<td>0.19 (0.02, 0.38)</td>
</tr>
<tr>
<td>$X_{\text{INSP}}$ vs. $X_{\text{INSP}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.55</td>
<td>0.88</td>
<td>0.58</td>
<td>0.61</td>
</tr>
<tr>
<td>Slope</td>
<td>$-0.12$ (−0.21, −0.03)</td>
<td>$-0.17$ (−0.33, −0.01)</td>
<td>$-0.14$ (−0.24, −0.04)</td>
<td>$-0.18$ (−0.30, −0.06)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Subjects with Asthma</th>
<th>Healthy Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{INSP}}$ vs. $R_{\text{INSP}}$</td>
<td>$R^2$</td>
<td>Slope</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>0.11 (0.04, 0.17)</td>
</tr>
</tbody>
</table>

$R_{\text{INSP-IQR}}$, interquartile range of $R_{\text{INSP}}$; $X_{\text{INSP-IQR}}$, interquartile range of $X_{\text{INSP}}$. See Table 2 for other abbreviations. Slope values are presented as means (95% confidence interval).
with an increase in transpulmonary pressure, and thereby bronchial transmural pressure, the present results suggest that strain is the major determinant of the bronchodilator effect of DI, whereas stress has a marginal effect in healthy subjects. This is in line with a recent study in ovine trachea showing that stress may in part compensate for the reduction of strain in preserving the effect of simulated Dls in isolated ASM (43). The present study, however, adds that this effect of stress is reduced more in people with asthma than in healthy individuals. It must be noted, however, that in more severe asthma, strain and stress may be reduced at the same time because of a loss of interdependence, thus more severely impairing the bronchodilator effect of DI.

The significant correlations between RINSPIR_IQR and RINSPIR_Slope in both groups and between XINSPIR_IQR and XINSPIR_Slope in subjects with asthma suggest that short-term fluctuations in airway caliber are related to ASM velocity of shortening. These findings extend the results of Salome et al. (47) who reported that airway renarrowing after DI as estimated from the changes in Rrs over time was faster in subjects with asthma than in healthy subjects, and that this was significantly correlated with the fluctuation in airway tone within tidal breaths. That the ASM velocity of shortening may be a major determinant of bronchial tone control within tidal breaths. That the ASM velocity of shortening may be a major determinant of bronchial tone control in asthma is also suggested by the significant correlation we observed between RINSPIR_Slope and XINSPIR_Slope after MCh, as a confirmation that when ASM shortening velocity is increased, it contributes not just to a faster reconstruction but also to a faster reappearance of airway closure. The lack of significance between XINSPIR_IQR and XINSPIR_Slope in healthy subjects is likely due to the small change in XINSPIR with MCh, consistent with the hypothesis of a more central airway narrowing than in asthma.

Conclusions

Altogether, the results of the present study confirm that the short-term variability in respiratory impedance is mostly related to the intrinsic properties of ASM and is increased by operating at short length, due to either unloading or contractile shortening. Nevertheless, there are differences in behavior between ASM in subjects with asthma and healthy subjects, consisting of an increased velocity of shortening and a reduced sensitivity to mechanical stress when strain is limited.

GRANTS

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DISCLOSURES


AUTHOR CONTRIBUTIONS


REFERENCES


