Effect of methacholine on peripheral lung mechanics and ventilation heterogeneity in asthma

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Downie SR, Salome CM, Verbanck S, Thompson BR, Berend N, King GG. Effect of methacholine on peripheral lung mechanics and ventilation heterogeneity in asthma. J Appl Physiol 114: 770–777, 2013. First published January 31, 2013; doi:10.1152/japplphysiol.01198.2012.—The forced oscillation technique (FOT) and multiple-breath nitrogen washout (MBNW) are noninvasive tests that are potentially sensitive to peripheral airways, with MBNW indexes being especially sensitive to heterogeneous changes in ventilation. The objective was to study methacholine-induced changes in the lung periphery of asthmatic patients and determine how changes in FOT variables of respiratory system reactance (Xrs) and resistance (Rrs) and frequency dependence of resistance (Rrs5-Rrs19) can be linked to changes in ventilation heterogeneity. The contributions of air trapping and airway closure, as extreme forms of heterogeneity, were also investigated. Xrs5, Rrs5, Rrs19, Rrs5-Rrs19, and inspiratory capacity (IC) were calculated from the FOT. Ventilation heterogeneity in acinar and conducting airways, and trapped gas (percent volume of trapped gas at functional residual capacity/vital capacity), were calculated from the MBNW. Measurements were repeated following methacholine. Methacholine-induced airway closure (percent change in forced vital capacity) and hyperinflation (change in IC) were also recorded. In 40 mild to moderate asthmatic patients, increase in Xrs5 after methacholine was predicted by increases in ventilation heterogeneity in acinar airways and forced vital capacity (r = 0.37, P < 0.001), but had no correlation with ventilation heterogeneity in conducting airway increase or IC decrease. Increases in Rrs5 and Rrs5-Rrs19 after methacholine were not correlated with increases in ventilation heterogeneity, trapped gas, hyperinflation, or airway closure. Increased reactance in asthmatic patients after methacholine was indicative of heterogeneous changes in the lung periphery and airway closure. By contrast, increases in resistance and frequency dependence of resistance were not related to ventilation heterogeneity or airway closure and were more indicative of changes in central airway caliber than of heterogeneity.

ventilation heterogeneity; forced oscillation technique; multiple-breath nitrogen washout; lung impedance

ABNORMALITY IN THE PERIPHERAL airways (less than ~2 mm diameter) is an important feature of asthma and is associated with nocturnal asthma (31), fatal asthma (3), recurrent exacerbations in severe asthma (18), symptoms (11), and response to inhaled corticosteroid (ICS) treatment (12). Peripheral airway structure and function in asthma have been investigated by means of frequency dependence of compliance (51), wedge bronchoscope techniques (50, 54), and gas trapping visualized in high-resolution computed tomography (56). Taken together, these studies suggest the presence of alterations in the small airways of asthma patients at baseline and after bronchoprovocation, stimulating the application of noninvasive measurement techniques that are potentially sensitive to small-airway function to investigate disease mechanisms in asthma (9, 47). This approach requires that the actual response of these noninvasive indexes to specific structural changes, such as increased airway obstruction or decreased airway compliance, is fully understood, including in the extreme case of airway closure.

The forced oscillation technique (FOT) is theoretically sensitive to peripheral airway function (26, 43) and is easy to administer because it is effort independent. Sinusoidal pressure oscillations are imposed at the airway opening, and the relationship between pressure and flow in response to the oscillating pressure reflects the mechanical properties of the respiratory system. Results of modeling studies suggest that parameters of respiratory system resistance (Rrs) and reactance (Xrs) are sensitive to changes in the heterogeneity of airway caliber. Heterogeneity of airway caliber can be classified as either parallel, comparing branches of the airway tree within the same generation, or serial, comparing branches of the airway tree between different generations. Overall heterogeneity describes both the parallel and serial components of heterogeneity of airway caliber. Xrs is particularly sensitive to parallel heterogeneity, closure (26, 43), and to static and dynamic compliance (36). In addition, heterogeneous narrowing with closure of some airways causes greater increases in Rrs and Xrs, compared with homogeneous narrowing of a similar magnitude (26). Therefore, there is potential for the FOT to detect homogeneous and heterogeneous changes in proximal and peripheral airways. Since lower frequencies are considered to represent resistance from large to peripheral airways, the frequency dependence of resistance, obtained by subtraction of the higher frequency resistance, e.g., Rrs at 5 Hz minus Rrs at 20 Hz, has also been regularly used as a more specific marker of peripheral airway function (52). Despite this, other reports indicate that frequencies <5 Hz need to be used to fully appreciate the small airway effect (27). Hence, the validity of the frequency dependence of resistance >5 Hz, as it is currently used in clinical research to measure peripheral airway function, is still controversial.

The multiple-breath nitrogen (N2) washout (MBNW) is a noninvasive method in which ventilation heterogeneity is measured from the pattern of N2 washout from the lung during tidal
breathing of 100% oxygen. Based on modeling two types of gas transport, diffusion and convection (4, 5, 38), ventilation heterogeneity is partitioned into regions of the most peripheral, diffusion-convection-dependent (Sanin) vs. the less peripheral, convection-dependent (Scond) airways. Increases in Sanin and asthma (49). Both Sanin (23) and Scond (23, 48) increase with treatment with fine- compared with coarse-particle ICS in peripheral airways associated with cigarette smoking (46) and are more sensitive than spirometry for detecting early changes in peripheral airways with cigarette smoking (46) and with treatment with fine- compared with coarse-particle ICS in asthma (49). Both Sanin (23) and Scond (23, 48) increase significantly following provocations with methacholine in healthy nonasthmatic patients. The key to the detection capabilities of MBNW is that the lung changes occur heterogeneously. In the conductive airways, an increased parallel heterogeneity in specific ventilation and flow asynchrony (with the best ventilated unit emptying first) will increase Scond. In the acinar airways, an increased asymmetry of the intra-acinar ramification pattern (e.g., by heterogeneous narrowing of airways) will increase Sanin.

While FOT variables reactance (Xrs), resistance (Rrs), and frequency dependence of resistance [Rrs at 5 Hz (Rrs5)-Rrs at 19 Hz (Rrs19)] are potentially sensitive to both overall and parallel heterogeneity of airway changes, MBNW variables Sanin and Scond are predominantly sensitive to parallel heterogeneity of airway changes. Hence, the hypothesis of this study was that, if parallel heterogeneity is an important feature of methacholine-induced challenge of the proximal and peripheral airways in asthma patients, there must be links between resulting changes in FOT- and MBNW-derived indexes. We assessed to what extent changes in FOT variables after methacholine challenge were also reflected by changes in MBNW variables, and by trapped gas or airway closure.

METHODS

Asthmatic Patients

Patients with asthma were recruited by advertising displayed throughout the University of Sydney and from the volunteers database at the Woolcock Institute of Medical Research. Written, informed consent was obtained from all patients, and the study was approved by the Central Sydney Area Health Service Ethics Review Committee. Inclusion criteria were doctor-diagnosed asthma (34) and asthma symptoms within the last 12 mo. Exclusion criteria for all asthmatic patients were any other lung disease apart from asthma, oral prednisone use in the last 4 wk, an upper respiratory tract infection in the last 4 wk, smoking in the last 6 mo, or a >10 pack-yr smoking history.

This study is registered on the Australian New Zealand Clinical Trials Registry (no. 012605000317695) and was approved by the Human Research Ethics Committee of the Sydney Central and South-Western Area Health Service. Some of the results of this study have been previously published (9) and reported in abstract form (8, 10).

Study Design

In a single visit, all patients underwent baseline FOT, spirometry, and MBNW, and these tests were repeated after methacholine challenge. Figure 1 shows the time points of all lung function testing at baseline and after methacholine. Standard MBNW involved three tests, totaling ~20-min duration, raising the concern of spontaneous reversal of bronchoconstriction (39) for the postmethacholine measurements. To address this, FOT was also measured at three similar time points, both before and after challenge, to be representative of function over the 20 min. These extra FOT measurements were recorded in between MBNW tests when patients were waiting for their alveolar N2 concentration to return to ambient levels. Since the protocol for some FOT and all MBNW measurements involved deep breaths (discussed further in the relevant sections below), and deep breaths are known to relieve bronchial tone after methacholine (42), it was also important to capture measurements of both FOT and MBNW at similar time points, particularly because the comparison between these tests is the primary outcome of the study. Bronchodilator was administered after the final postmethacholine FOT and MBNW measurements. Airway closure was measured indirectly by the percent change in forced vital capacity (FVC) (14, 33, 41, 55) after methacholine challenge, and by gas trapping at functional residual capacity (FRC) measured from the MBNW (16) before and after methacholine. Hyperinflation was estimated from change in inspiratory capacity (IC) after methacholine challenge.

FOT

FOT was measured using a combined 5-, 11-, and 19-Hz oscillation signal optimized for signal-to-noise ratio. Data were acquired during a single 60-s period of tidal breathing, with the patients supporting their cheeks with their hands, while wearing a nose clip. IC was measured from a slow, deep inspiration performed at the end of the tidal breathing. Measurements performed in between MBNW washouts did not include the IC maneuver. The FOT device was developed in-house, as previously described (44). Briefly, flow was measured by a 50-mm-diameter Fleisch type pneumotachograph and a ±2.5-cmH2O solid state pressure transducer with a range of ±12.5 cmH2O. The pressure and flow signals were measured and processed, as described in detail previously (7, 44), to calculate Rrs and Rrs19 and reactance at 5 Hz (Xrs5). The frequency dependence of resistance was quantified by the difference (Rrs5-Rrs19). Any extreme outliers were automatically identified and removed, as previously described (44).

MBNW

The MBNW was performed as previously described (9). A closed-circuit, bag-in-box breathing system delivered 100% O2 during inspiration with separate capture of exhaled breath. N2 concentration was measured at the mouth by a model 721 KaeTech Nitrogen Analyzer.
Sn of first breath

the contribution of conductive airway ventilation heterogeneity (mean

the regression. Sacin was derived from the Sn of the first breath minus

patients had airway hyperresponsiveness. Nineteen patients

FOT measurements recorded in the 20 min immediately following

measurements that were made at baseline and postmethacholine

the alveolar phase III slope of N2 concentration vs. expired volume of

each breath was divided by the corresponding mean expired N2

Sacinda and Scond were calculated from the MBNW as follows. Each

mean lung turnover of 1st breath).

W = PndNd × mean lung turnover of 1st breath).

For the MBNW calculation of trapped gas at FRC, the patient took

five slow deep breaths between FRC and total lung capacity imme-

diately after mean expired N2 concentration dropped to <2%. This

allowed for the washout of any remaining N2 from areas of unventi-

lated or severely underventilated lung during breathing from FRC to

FRC + 1 liter. The volume of trapped gas was calculated (VtrFRC) as

described by Gustafsson et al. (16), and was reported as %VtrFRC/

VC. VC was calculated from a final normal inhalation and then

exhalation to residual volume after completion of the five slow deep

breaths at the end of the MBNW.

Sacin and Scond were calculated from the MBNW as follows. Each

breath of all three MBNW tests were analyzed individually, whereby

the alveolar phase III slope of N2 concentration vs. expired volume of
each breath was divided by the corresponding mean expired N2

correlation

also taking long-acting β2-agonist as part of their combination

therapy.

Changes in MBNW and FOT Variables After Methacholine

Challenge

The mean percent fall in FEV1 following challenge was 21.6 ± 9.6%, with a range of −0.5–40% after a mean dose of

2.9 ± 2.5 μmol methacholine. The mean percent fall in FVC was 14.2 ± 9.1% with a range of −2.7–37.9%. Metha-

choline challenge induced significant (P < 0.01) changes in all MBNW and FOT variables (Table 2). The increases (i.e.,
increasing ventilation heterogeneity) in Scond and Sacin following methacholine were unrelated (rs = −0.09, P = 0.59);

however, the increase in Rrs5 and increase in Xrs5 were correlated (rs = 0.49, P < 0.01), as were the increases in Rrs5

and Rrs5-Rrs19 (rs = 0.9, P < 0.0001) and the increases in Rrs5-Rrs19 and Xrs5 (rs = 0.66, P < 0.0001). The percent
decrease in FVC was significantly correlated with the increase in %VtrFRC/VC (rs = 0.64, P < 0.0001) after methacholine.

There were no significant differences in the three postmethacholine FOT recordings (for Rrs5: F statistic = 0.11, P = 0.9; for

Xrs5: F statistic = 0.6, P = 0.5; for Rrs5-Rrs19: F statistic = 0.37, P = 0.7).

Predictors of increase in reactance (Xrs5) after methacholine

challenge. The increase in Xrs5 from the FOT after methacholine (i.e., it becoming more negative) was signifi-
cantly correlated with the increase (i.e., worsening) in Sacin from the MBNW (rs = 0.54, P < 0.01, Fig. 2), the increase in

airway hyperinflation (IC: rs = 0.24, P = 0.13). In multiple linear regression, the increase in Xrs5 after methacholine

challenge was predicted by both the increase in Sacin and percent decrease in FVC (r2 = 0.40, P < 0.0001, Fig. 5). The

partial r2 for Sacin was 0.32 (P < 0.001; β-coefficient = 14, 95% confidence interval = 6–22), and for percent decrease in

FVC was 0.08 (P = 0.04, β-coefficient = 0.05, 95% confidence interval = 0.003–0.095).

Predictors of increase in Rrs5 and Rrs19 after methacholine

challenge. The increase in Rrs5 after methacholine challenge was not related to the increases in Scond (rs = −0.01, P = 0.94) or hyperinflation (IC: rs = 0.24, P = 0.13).

RESULTS

Baseline Patient Characteristics

Baseline characteristics of the 40 patients with asthma who participated in this study are summarized in Table 1. The mean

percent predicted FEV1 was 82.2 ± 11.5%, and 32 of 40 patients had airway hyperresponsiveness. Nineteen patients

were taking ICS treatment regularly, 10 of these patients were

Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Asthmatic Patients</th>
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</thead>
<tbody>
<tr>
<td>Age (range), yr</td>
</tr>
<tr>
<td>Male/female</td>
</tr>
<tr>
<td>AHR, yes/no</td>
</tr>
<tr>
<td>%Predicted FEV1</td>
</tr>
<tr>
<td>%Predicted Rrs5</td>
</tr>
<tr>
<td>β-Agonist use, occasions/day</td>
</tr>
<tr>
<td>BDP equivalent ICS dose, mg</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 40. AHR, airway hyperresponsiveness; FEV1, forced expiratory volume in 1 s; Rrs5, respiratory system resistance at 5 Hz; BDP, beclomethasone dipropionate; ICS, inhaled corticosteroid.

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The increase in Rrs19 after methacholine challenge was not related to the increases in Scond (rs = 0.28, P = 0.08), Sacin (rs = 0.10, P = 0.52), or %VtrFRC/VC (rs = 0.10, P = 0.052) or percent decrease in FVC (rs = 0.19, P = 0.24).

Predictors of increase in the frequency dependence of resistance (Rrs5-Rrs19) after methacholine challenge. The increase in the frequency dependence of resistance (Rrs5-Rrs19) after methacholine challenge was not related to the increases in Sacin (rs = 0.20, P = 0.21), Scond (rs = 0.23, P = 0.15), or %VtrFRC/VC (rs = 0.18, P = 0.28), or percent decrease in FVC (rs = -0.03, P = 0.85) or percent decrease in FVC (rs = 0.10, P = 0.55).

DISCUSSION

In this study, we have shown that the peripheral airway response to inhaled methacholine in patients with asthma was evident by changes in both FOT and MBNW parameters. The increase in reactance (Xrs5) after methacholine correlated independently with the increase in both ventilation heterogeneity in the most peripheral lung zone, represented by Sacin, and airway closure (FVC). In contrast, the increase in resistance (Rrs5 and Rrs19) after methacholine challenge, including frequency dependence of resistance, was unrelated to any MBNW parameters. These findings suggest that the response to methacholine challenge in asthmatic patients occurring at the lung periphery, more specifically at the level of the diffusion front, was only associated with the changes in Xrs5, but was not associated with change in the resistance parameters of the FOT. Moreover, increases in reactance were associated with increase in %VtrFRC/VC and percent decrease in FVC, suggesting that at least part of this very peripheral response in patients with asthma was determined by gas trapping and airway closure.

Findings in the present study of a peripheral airway response to inhaled methacholine in asthmatic patients are consistent with previously published studies involving single-breath N2 washout (13), wedged bronchoscopes (19, 20), and wedged catheter studies (35). Peripheral airway responses were also

Table 2. Changes in spirometry, MBNW, and FOT variables after methacholine

<table>
<thead>
<tr>
<th>Spirometry</th>
<th>Asthmatic Patients</th>
<th>Absolute Change</th>
<th>%Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1, liter</td>
<td>2.95 ± 0.7</td>
<td>-0.7 ± 0.3</td>
<td>-21.6 ± 9.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FVC, liter</td>
<td>3.93 ± 0.9</td>
<td>-0.56 ± 0.4</td>
<td>-14.2 ± 9.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>0.75 ± 0.1</td>
<td>-0.08 ± 0.1</td>
<td>-10.4 ± 7.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FEF25–75%, l/s</td>
<td>2.50 ± 0.9</td>
<td>-0.91 ± 0.6</td>
<td>-35.9 ± 16.5</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MBNW</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Sacin, liter⁻¹</td>
<td>0.148 ± 0.05</td>
<td>+0.054 ± 0.06</td>
<td>+36.9 ± 45.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Scond, liter⁻¹</td>
<td>0.057 ± 0.03</td>
<td>+0.039 ± 0.03</td>
<td>+67.3 ± 245.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>%VtrFRC/VC</td>
<td>3.5 ± 2.4</td>
<td>+6.3 ± 5.6</td>
<td></td>
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</tr>
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</table>

FOT

| Rrs5, cmH2O·1⁻¹·s²   | 4.7 ± 1.6           | +2.1 ± 1.7      | +47.5 ± 35.1 | <0.01   |
| Rrs19, cmH2O·1⁻¹·s²  | 4.0 ± 1.2           | +0.7 ± 0.8      | +19.6 ± 17.1 | <0.01   |
| Xrs5, cmH2O·1⁻¹·s²   | -1.8 ± 1.2          | -2.1 ± 1.6      | <0.01       |
| Xrs5-Rrs19, cmH2O·1⁻¹·s² | 0.7 ± 0.7          | +1.3 ± 1.3      | <0.01       |
| IC, liter            | 2.41 ± 0.6          | -0.4 ± 0.4      | -16.4 ± 15.7 | <0.01   |

Values are means ± SD; n = 40. FVC, forced vital capacity; FEF25–75%, 25–75% forced expiratory flow; MBNW, multiple-breath nitrogen washout; Sacin, ventilation heterogeneity in acinar airways; Scond, ventilation heterogeneity in conducting airways; %VtrFRC/VC, percent volume of trapped gas at functional residual capacity/vital capacity; FOT, forced oscillation technique; Xrs5, respiratory system reactance at 5 Hz; IC, inspiratory capacity.

Fig. 2. The increase in reactance [respiratory system reactance at 5 Hz (Xrs5)] after methacholine was significantly correlated with the increase in acinar ventilation heterogeneity (Sacin), (rs = 0.54, P < 0.001).

Fig. 3. The increase in Xrs5 after methacholine was significantly correlated with airway closure [%decrease in forced vital capacity (FVC); rs = 0.43, P = 0.01].
found in a study using MBNW in healthy patients who underwent high-dose methacholine challenge (23). Although the peripheral airways respond to methacholine inhalation in both healthy and asthmatic patients, there is a more heterogeneous response in asthmatic patients (17, 22, 40, 45), which is reflected in impedance and gas washouts from the present study. However, the more heterogeneous response to methacholine in asthmatic patients compared with healthy patients may not occur when the patients are supine (21). The reason for more heterogeneous narrowing in asthmatic compared with nonasthmatic patients is unknown, but there could be variability in structure and function along airway paths and between airways of similar generation due to inflammation and remodeling. The presence of increased ventilation heterogeneity in clinically stable asthmatic patients at baseline, who had near normal lung function, is consistent with this (9, 24, 47).

The results of computational modeling studies suggest that FOT parameters are sensitive to the presence of heterogeneous and widespread peripheral airway narrowing, accompanied by closure or near closure of some more proximal airways. Thorpe and Bates (43) found that the time course of changes in dynamic elastance and tissue resistance induced by histamine in canine lungs was due to heterogeneous peripheral airway narrowing and closure, as opposed to homogeneous narrowing. The simulations of Lutchen and Gillis (26) also used a canine lung model to show that frequency dependence of both lung resistance and elastance was sensitive to peripheral airway narrowing that was widespread and heterogeneous and also associated with more proximal airway closure. In both of these simulations, airway closure was inherent and critical to explaining the typical changes that occurred in lung resistance and elastance. Confirmation that reactance is sensitive to airway closure has been shown in an allergic mouse model (25) and in a pig model of acute lung injury (6). The findings in the present study that changes in reactance are sensitive to increased ventilation heterogeneity in the lung periphery and to airway closure are thus consistent with the previously published data.

The FOT parameter $R_{rs5}-R_{rs19}$ [similar to $R_{5}-R_{20}$ reported elsewhere (52)] has been suggested as a marker of the peripheral airways, based on model predictions by Otis et al. (37) that frequency dependence of both resistance and compliance should occur in diseased lungs when there are heterogeneities in time constants. Grimby et al. (15) subsequently demonstrated frequency dependence of $R_s$ at 3, 5, 7, and 9 Hz in chronic obstructive pulmonary disease and asthma, when healthy patients were shown to have no frequency dependence. Our data show that, although $R_{rs5}-R_{rs19}$ does change with methacholine challenge, these changes were unrelated to changes in any of the MBNW variables under study. In particular, the correlations between the methacholine-induced increase in $R_{rs5}$ or $R_{rs19}$ and $S_{cond}$ were close to significance, but the correlation of $R_{rs5}-R_{rs19}$ and $S_{cond}$ was not. Since frequency dependence of resistance is thought to be most sensitive to peripheral heterogeneity at frequencies lower than 5 Hz (2, 27, 29), and 5 Hz is well above the normal breathing frequency of 0.2–0.6 Hz, $R_{rs5}-R_{rs19}$ could, in fact, represent...
changes in airway calibers in airways larger than those that influence the most peripheral MBNW indexes (i.e., $S_{\text{acin}}$). In more proximal airways, $S_{\text{cond}}$ is potentially sensitive to heterogeneity of caliber in conductive airways of all sizes, the absence of correlation between $S_{\text{cond}}$ and $R_{RS_{5-19}}$ suggests that the latter (and indeed $R_s$ in the frequency range used in the present study) is more sensitive to mean airway caliber than to heterogeneity of caliber.

Two previous studies (28, 30) found no association between changes in impedance and MBNW variables following bronchodilation in patients with asthma. This could suggest that impedance and MBNW parameters are complementary in describing peripheral vs. central lung mechanics. However, it is difficult to directly compare these studies with the present study, since they involved a smaller number of patients, used global measures of heterogeneity, which did not differentiate between the conducting and more peripheral acinar airways, and used different methods for collecting impedance data. Moreover, it is possible that induced bronchodilation and bronchoconstriction differ in both the degree and site of response and may not be directly comparable. The same holds when considering differing provoking agents. For instance, Michils et al. (32) found that, in steroid-naive asthma patients, adenosine 5’-monophosphate elicited a more marked increase in the single-breath He phase III slope than methacholine, for a similar $SF_6$ phase III slope increase with both agents. While this clearly signals a greater response in the proximal acinar airways to adenosine 5’-monophosphate than to methacholine, it was also suggested that the predominant effect of methacholine was probably in the conductive airways. While the present study shows generally smaller increases in phase III slopes (from which $S_{\text{acin}}$ and $S_{\text{cond}}$ are derived) for a similar $FEV_1$ decrease as in Michils et al. (32), the relatively greater $S_{\text{cond}}$ than $S_{\text{acin}}$ increase with methacholine (Table 2) is also suggestive of an important effect in the conductive airways. However, the observed methacholine-induced $S_{\text{cond}}$ changes do not appear to be related to any of the FOT-derived indexes sensitive to changes in the conductive airways.

In previous studies, change in FVC has been suggested to be an indirect marker of airway closure (14, 33, 41, 55), and our finding in the present study of a solid relationship between percent change in FVC and increase in percentage of trapped gas (%VtrFRC/VC) at FRC (17) after methacholine supports this argument. However, we cannot rule out the possibility that at least some of the percent decrease in FVC could be due to severely underventilated, rather than totally closed, regions of the lung. Methacholine-induced bronchoconstriction in the present study could be affected by both spontaneous reversal during the course of the 20-min postchallenge MBNW measurement (39), and/or attenuation from deep breaths in the breathing protocols (42). We have best addressed these issues by including repeated FOT recordings in between the individual MBNW tests, and reporting the primary outcome variables as the mean of these three FOT and MBNW tests. Furthermore, since each of the three FOT recordings after methacholine could be compared individually over the 20-min period, our finding that FOT variables did not change significantly in that time indicates that the effect of deep breaths and spontaneous reversal of bronchoconstriction were likely to be negligible.

In summary, we have found a significant relationship between methacholine-induced changes in reactance at 5 Hz and $S_{\text{acin}}$ in patients with asthma. Reactance is sensitive to heterogeneous changes in the lung region from the point where diffusion-convection-dependent interactions occur, i.e., from about the acinar entrance and down, and where ventilation heterogeneity, airway closure, and gas trapping are likely to occur. The relationship between changes in resistance and conducting airway heterogeneity was only marginal, deserving of further investigation, perhaps at lower oscillation frequencies. We conclude that methacholine-induced changes in reactance were indicative of heterogeneous changes in the lung periphery and airway closure, while changes in resistance and its frequency dependence $>5$ Hz could be more indicative of changes in central airway caliber than of heterogeneity. Therefore, MBNW and FOT are complementary in describing peripheral and central lung mechanics following methacholine.

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DISCLOSURES

C. M. Salome has been reimbursed by AstraZeneca for participating as a speaker at symposia organized by the company. B. R. Thompson received an unrestricted research grant from Pharmaxis, which has also funded a number of research grants for participating in multicentre trials. N. Berend has served on advisory boards for Boehringer Ingelheim, Pfizer, GlaxoSmithKline, AstraZeneca, and Bayer with no personal benefit. N. Berend has received $1,000 payment for speaking at a symposium for Pfizer. G. G. King has received travel sponsorship from GlaxoSmithKline to attend the ATS ASM 2003 (approximately $AUS$9,000) and a GlaxoSmithKline meeting in 2003 (approximately $AUS$10,000), travel sponsorship from AstraZeneca to attend ATS ASM 2004 (approximately $AUS$10,000) and two 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 (SAUS$3,000) in 2005, and a travel grant from AstraZeneca to attend the ATS 2005 (SAUS$6,000). A proportion of G. G. King’s research work is conducted at the Woolcock Institute of Medical Research, which receives unrestricted grants from AstraZeneca and GlaxoSmithKline, and which also has a consultancy agreement with Pfizer, Boehringer Ingelheim, AstraZeneca, and GlaxoSmithKline, for which Dr. King provides consultancy services related to asthma and chronic obstructive pulmonary disease. His research group receives a proportion of the grants and monies that arise from those companies, as part of a general allocation of those funds for research purposes across all research groups of the Woolcock Institute of Medical Research. S. R. Downie and S. Verbanck have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS


REFERENCES


