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Modulation of airway caliber by deep inhalation in children

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Milanese, Manlio, Chiara Mondino, Mariangela Tosca, G. Walter Canonica, and Vito Brusasco. Modulation of airway caliber by deep inhalation in children. *J Appl Physiol* 88: 1259–1264, 2000.—To elucidate whether deep inhalation (DI) modulates changes in airway caliber in childhood, we measured the effect of DI on respiratory impedance before and after inhaled methacholine or salbutamol in 4- to 7-yr-old children ($n = 15$) suffering from recurrent wheezing. In all children, the real part of impedance between 12 and 16 Hz ($Re[Z]_{12–16}$) increased after methacholine from 5.6 ± 1.2 to 8.2 ± 1.6 $cmH_2O \cdot l^{-1} \cdot s$ ($P < 0.001$) and resonance frequency from 18 ± 3 to 25 ± 5 Hz ($P < 0.001$). These changes were partially reversed by DI: $Re[Z]_{12–16}$ decreased to 7.2 ± 1.2 $cmH_2O \cdot l^{-1} \cdot s$ ($P < 0.01$) and resonance frequency to 19 ± 5 Hz ($P < 0.001$). In nine children, on a separate occasion, $Re[Z]_{12–16}$ decreased after salbutamol from 8.3 ± 1.9 to 5.1 ± 0.9 $cmH_2O \cdot l^{-1} \cdot s$ ($P < 0.001$) and resonance frequency from 21 ± 6 to 15 ± 3 Hz ($P < 0.05$). The decrease of $Re[Z]_{12–16}$ was partially reversed by DI (to 6.2 ± 1.4 $cmH_2O \cdot l^{-1} \cdot s$, $P < 0.01$), but resonance frequency did not change significantly ($P = 0.75$). We conclude that in 4- to 7-yr-old children pharmacologically induced changes in airway caliber are modulated by DI. These findings suggest that airway-to-parenchyma interdependence is operative in this age range.

respiratory resistance; resonance frequency; bronchoconstriction; bronchodilatation; forced-oscillation technique; wheezing

IN BOTH HEALTHY AND MILDLY ASTHMATIC adults, deep inhalation (DI) causes bronchodilatation during induced bronchoconstriction (8, 22) and bronchoconstriction after inhalation of usual doses of bronchodilators (1, 35). These volume-history effects are the result of airway-to-parenchyma interdependence, and their direction and magnitude are, according to a well-known theory (10), deemed to reflect the relationship between airway and parenchymal hystereses. When airway hysteresis increases, e.g., during airway smooth muscle contraction, DI would cause transient bronchodilatation unless parenchymal hysteresis increases concomitantly by the same amount. Conversely, when airway hysteresis decreases, e.g., during airway smooth muscle relaxation, DI would cause transient bronchoconstriction unless parenchymal hysteresis decreases concomitantly by the same amount.

In infants, Hayden and co-workers (12) reported that methacholine-induced bronchoconstriction was not attenuated by DI delivered by a raised-volume forced-expiration technique. This might be interpreted as suggesting that methacholine caused similar increments in airway and parenchymal hystereses or that the distending force of lung parenchyma is not sufficient to dilate constricted airways in infants. During the first few years of life, the number of alveoli increases and the connective tissue of the lung develops (2). Although data on lung mechanics before age 7 yr are lacking (6), it is conceivable that the above-mentioned changes in lung parenchyma result in a progressive increase of lung elastic recoil. If this is the case, the ability of DI to dilate the airways should also increase during growth. Indeed, a bronchodilator effect of DI on baseline airway caliber was reported in 8- to 10-yr-old children, although this effect was inconsistent between genders (15). To the best of our knowledge, whether DI modulates induced bronchoconstriction and bronchodilatation in preschool or primary school children has not been determined.

In this study, the effects of DI on airway caliber at baseline and during induced bronchoconstriction or bronchodilatation were investigated in a group of 4- to 7-yr-old children referred to our laboratory for recurrent wheezing (20). The forced-oscillation technique, which requires minimal cooperation and no skill in performing respiratory maneuvers, was used to infer changes in airway caliber.

METHODS

Subjects. The study was conducted on 15 outpatients (9 boys, 6 girls, age 5.6 ± 1.1 yr, weight 23.3 ± 5.4 kg, height 120 ± 8 cm; means ± SD) with history of recurrent wheezing. Six children were allergic to house dust mite, as determined by skin prick test. None of them was taking antiasthmatic treatments other than short-acting β-adrenoceptor agonists on an as-necessary basis. To enter the study, the subjects were required not to have suffered from exacerbations of their disease in the previous month and to abstain from bronchodilators for 24 h before the study. Informed consent was obtained from the parents, who attended the studies.

Measurements. Respiratory input impedance was measured by a forced-oscillation wave-tube technique as previ-

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ously described (3). In this system, the excitation signal is generated by a 12-in. loudspeaker driven by multi-sine frequency random noise (33). A wave tube consisting of 28 pipelets (15-cm long, 2-mm ID) arranged in parallel is placed in series between the loudspeaker and the subject. Absolute pressures across the wave tube are measured by using two identical pressure transducers (Setra 239, ± 2 cmH_2O). A bias flow continuously enters the system between the loudspeakers and the wave tube. A wide-bore (2-m long, 1-in. ID) tube behaving as a low-pass shunt impedance is placed near the mouthpiece. This configuration allows the low-frequency components of spontaneous breathing to be shunted to the atmosphere and to reduce the load imposed on the subject's respiratory system by the relatively high impedance of the wave tube. The signals from the pressure transducers are band-pass filtered (2-Hz high-pass filter with 12-dB/octave slope and 80-Hz antialiasing low-pass filter with 36-dB/octave slope) and sampled at 256 Hz. Four thousand ninety-six samples for input and output pressures are recorded over 16 s, divided into 31 blocks of 256 data each with 50% overlap. For each block, the fast Fourier transform is calculated after Hanning windowing. Respiratory impedance and coherence function are obtained by using average auto- and cross spectra. The system was calibrated before each study by using a mechanical analog consisting of a brass tube filled with a bundle of 30 pipelets (20-cm long, 2-mm ID) and Araldite glue between pipelets. The predicted impedance values of this analog have a real part (Re[Z]) ranging from 3.02 $cmH_2O \cdot l^{-1} \cdot s$ at 2 Hz to 3.87 $cmH_2O \cdot l^{-1} \cdot s$ at 48 Hz and an imaginary part (Im[Z]) ranging from 0.47 $cmH_2O \cdot l^{-1} \cdot s$ at 2 Hz to 9.96 $cmH_2O \cdot l^{-1} \cdot s$ at 48 Hz (5).

Measurements were taken while children were sitting with head in a natural position and cheeks and mouth floor supported by their hands. Data were accepted only if the coherence function was ≥0.95. Respiratory resistance was inferred from the Re[Z] values between 12 and 16 Hz ($Re[Z]_{12-16}$). The frequency of resonance was determined by third- or fourth-order polynomial fitting of Im[Z] vs. frequency.

Respiratory movements were monitored by respiratory inductive plethysmography (Respitrace) to ensure that the child did not sigh or take deep breaths when not requested, to check for the magnitude of DI, and to verify that data acquisition for impedance measurement was started after tidal breathing at functional residual capacity (FRC) had resumed. Rib cage and abdominal transducers were held in place by elastic bands positioned around the body at the levels of nipples and umbilicus. A single-posture method (30) was used for calibration of thoracic and abdominal signals over 30–50 spontaneous breaths. Signals from rib cage and abdomen were processed in direct-current mode and summed to obtain changes in lung volume, which were continuously displayed on a screen of a Gould Windograph recorder.

Measurements of respiratory input impedance were taken before and immediately after a single acceptable DI maneuver (i.e., an inspiration greater than twice the tidal volume) at control and after inhalation of a methacholine dose sufficient to increase $Re[Z]_{12-16}$ by more than 30% from control or 15 min after salbutamol (200 μg by metered dose inhaler and spacer).

Methacholine challenge. Solutions of methacholine were prepared by adding distilled water to dry powder methacholine chloride (Laboratorio Farmaceutico Lofarma, Milan, Italy). An SM-1 Rosenthal breath-activated dosimeter (SensorMedics, Yorba Linda, CA) driven by compressed air (30 psi) with 1-s actuations was used to deliver aerosols (10 μl per actuation) during quiet tidal breathing. After 20 inhalations of saline as a control, the subjects inhaled double-increasing doses of methacholine from 10 μg until $Re[Z]_{12-16}$, measured 1 min after each dose, increased by more than 30% of control. The double increments of dose were obtained by using two methacholine concentrations (1 and 10 mg/ml) with appropriate numbers of breaths. A 3-min interval was allowed between dose increments. At the end of the methacholine challenge, the subjects were given 200 μg of salbutamol and dismissed only when $Re[Z]_{12-16}$ had returned within 10% of control value. No complications or respiratory discomfort occurred during the challenges.

Data analysis. The effects of DI were evaluated only when $Re[Z]_{12-16}$ had changed by more than 30% in response to either methacholine or salbutamol. This value corresponded to more than twice the 95% upper confidence limit of the short-term intraindividual variability that we had previously determined in children of this age (14% or 0.93 $cmH_2O \cdot l^{-1} \cdot s$). To compare data from different individuals, an index of reversibility (RI), which is the ratio of the actual over the expected maximal bronchodilator effect of DI (36), was calculated as

$$RI = \frac{1/Re[Z]_{12-16}DI_{BC} - 1/Re[Z]_{12-16BC}}{1/Re[Z]_{12-16}DI_{CL} - 1/Re[Z]_{12-16BC}}$$

where the index DI refers to measurements taken after DI and the subscripts BC and CL indicate bronchoconstriction and control states, respectively.

Two-factor repeated-measure ANOVA with Duncan post hoc test was used to test the changes induced by DI at control and after methacholine or salbutamol for significance. The effects of age and gender were assessed by submitting the values of RI to linear regression analysis and unpaired Student's *t*-test, respectively. *P* values <0.05 were considered statistically significant. Data are presented as means ± SD.

RESULTS

All subjects did respond to inhalation of methacholine (20 to 600 μg, geometric mean 65.6 μg) with an increase of $Re[Z]_{12-16} > 30\%$ ($P < 0.001$), which was associated with a significant ($P < 0.001$) increase of the resonance frequency (Fig. 1). After DI, $Re[Z]_{12-16}$ increased slightly (from 5.6 ± 1.2 to 6.2 ± 1.2 $cmH_2O \cdot l^{-1} \cdot s$, $P < 0.05$) at control but decreased (from 8.2 ± 1.6 to 7.2 ± 1.2 $cmH_2O \cdot l^{-1} \cdot s$, $P < 0.01$) during bronchoconstriction (Fig. 1*A*). The resonance frequency was not significantly affected by DI at control (from 18 ± 3 to 17 ± 4 Hz, $P = 0.2$) but was reduced (from 25 ± 5 to 19 ± 5 Hz, $P < 0.001$) after DI during bronchoconstriction (Fig. 1*B*). The effects of methacholine and DI on mean impedance curves are shown in Fig. 2.

Mean RI values were significantly different from zero in both genders but greater in girls than in boys (0.72 ± 0.39 vs. 0.29 ± 0.32; $P < 0.05$), indicating greater bronchodilator effect of DI in the former. No significant relationship was found between RI and age ($r = 0.04$; $P = 0.88$).

On a separate occasion, 9 of the 15 children (6 boys, 3 girls, age 5.5 ± 1.0 yr, weight 21.7 ± 4.5 kg, height 119 ± 7.3 cm) responded to inhalation of salbutamol with a decrease of $Re[Z]_{12-16} > 30\%$ of control ($P < 0.001$), which was associated with a significant ($P < 0.05$) decrease of resonance frequency (Fig. 3). After DI, $Re[Z]_{12-16}$ decreased (from 8.3 ± 1.9 to 7.5 ± 2.1 $cmH_2O \cdot l^{-1} \cdot s$, $P < 0.05$) at control but increased (from

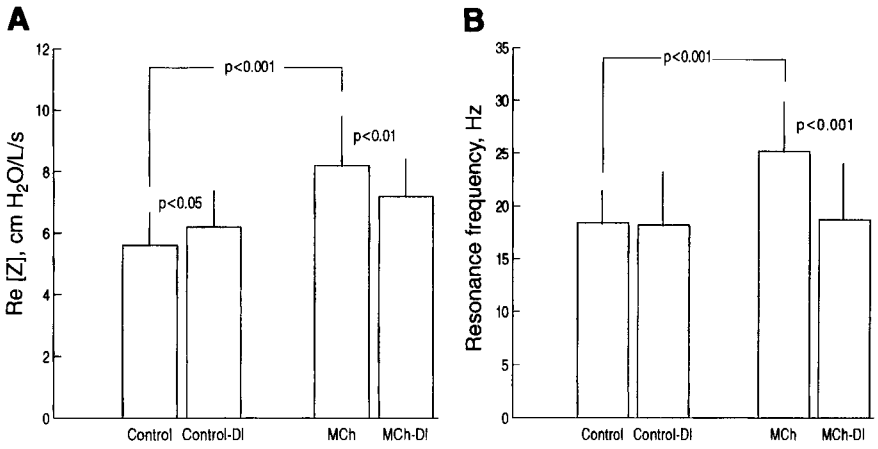

Fig. 1. Effects of methacholine (MCh) and deep inhalation (DI) on real part of respiratory impedance (Re[Z]) between 12 and 16 Hz (*A*) and resonance frequency (*B*). Data are means ± SD; $n = 15$.

5.1 ± 0.9 to 6.2 ± 1.4 $cmH_2O \cdot l^{-1} \cdot s$, $P < 0.01$) during bronchodilatation (Fig. 3*A*). The resonance frequency (Fig. 3*B*) was not significantly affected by DI either at control (from 21 ± 6 to 19 ± 6 Hz, $P = 0.18$) or after salbutamol (from 15 ± 3 to 14 ± 4 Hz, $P = 0.75$). The effects of salbutamol and DI on mean impedance curves are shown in Fig. 4.

DISCUSSION

The results of this study show that DI can modulate pharmacologically induced bronchoconstriction and bronchodilatation in 4- to 7-yr-old children. We interpret these data as suggesting that in this age range the force of interdependence between airways and lung parenchyma is already operative.

Comments on methodology. In this study, the effects of DI on airway caliber were inferred from changes in respiratory input impedance. The forced-oscillation technique used for this purpose has the advantage of being well tolerated and applicable in children with minimal cooperation (29), but it does not provide a direct measurement of airway resistance (29). Furthermore, measurements of Re[Z] are affected by breathing-related noise and upper airway shunting (29). The frequency range (12–16 Hz) over which Re[Z] measurements were taken seems to be relatively free from these artifacts in children (18) and less influenced by lung or chest wall resistances (29), thus approximating airway resistance.

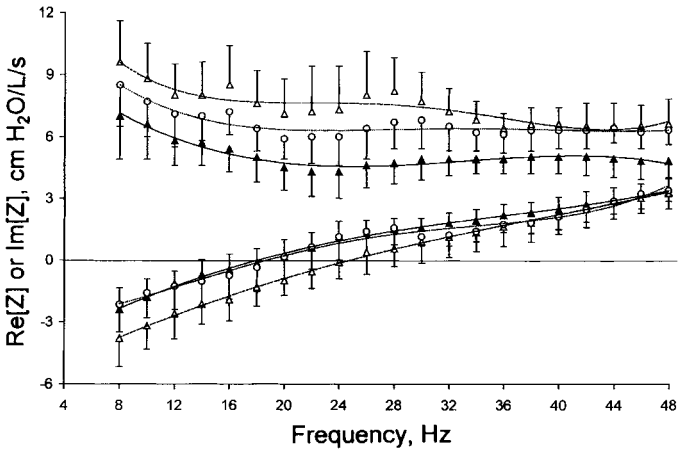

Fig. 2. Re[Z] (*top curves*) and imaginary part of respiratory impedance (Im[Z]; *bottom curves*) at control (▲, solid lines), after methacholine (△, dashed lines), and after DI (○, dotted lines). Data points are means ± SD, $n = 15$; lines were obtained by 4th-order polynomial fitting of mean values. Note that DI partially reversed effects of methacholine on both Re[Z] and resonance frequency.

During induced bronchoconstriction, the FRC may increase because of laryngeal constriction (14), dynamic hyperinflation (25), or both. Had an increase in FRC occurred after methacholine, both Re[Z] and Im[Z] would have been affected. In the present study, however, there were no changes in end-expiratory level that could be detected by the inductive plethysmograph. This lack of increase in FRC after methacholine was likely related to the mild degree of induced bronchoconstriction.

The criteria for grading airway obstruction or its reversibility by forced-oscillation technique are not standardized (16, 34). On the basis of intrasubject short-term variability of this measurement that we and others (37) have found in children of this age, a >30% change in $Re[Z]_{12-16}$ seems to be an appropriate cutoff for detecting significant bronchoconstriction or bronchodilatation.

The conventional ways to evaluate the effects of DI on airway caliber in adults have been comparisons of flows at the same lung volume on maximal and partial forced expiratory maneuvers (M/P ratio) or measurements of airway conductance (sGaw) or pulmonary resistance (R_L) before and after DI. In adults, sGaw and R_L return to pre-DI values in 1–2 min (24, 27). The forced-oscillation technique used for this study required an acquisition time of 16 s, during which DI-induced changes in airway caliber were likely reversed in part. It is therefore possible that changes in $Re[Z]_{12-16}$ underestimate the effects of DI on airway caliber compared with M/P ratio or changes in R_L or sGaw measured breath by breath (24, 26, 27). Furthermore, the computation of impedance on data blocks spanning several breath cycles does not allow estimation of the effect of differences in airway geometry between inspiration and expiration. These differences are likely to occur during tidal breathing in expiratory flow limitation, because of dynamic airway compression, and they may affect both parts of respiratory input impedance (28). In

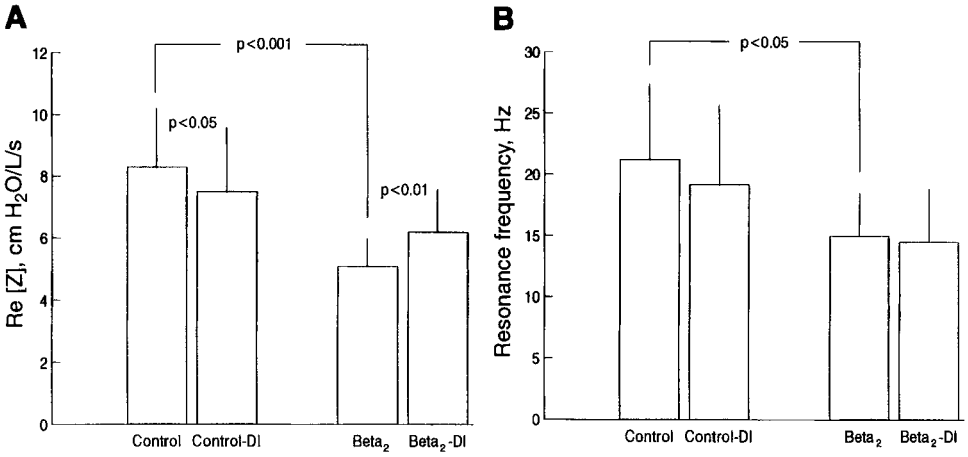

Fig. 3. Effects of salbutamol ($Beta_2$) and DI on Re[Z] between 12 and 16 Hz (*A*) and resonance frequency (*B*). Means ± SD, $n = 9$.

this study, however, expiratory flow limitation was unlikely to occur during tidal breathing because of the mild degree of bronchoconstriction.

For ethical reasons, only children with recurrent wheezing were studied and the degree of induced bronchospasm was mild. These limitations do not invalidate our conclusions, even though it cannot be excluded that the effect of DI might be different in more severely asthmatic children or during greater degrees of induced bronchoconstriction.

Comments on results. According to a theoretical analysis by Froeb and Mead (10), DI has an effect on airway caliber that depends on the ratio between airway and parenchymal hystereses: when the former prevails DI causes transient bronchodilatation, when the latter prevails DI causes transient bronchoconstriction. Mechanically, these changes would be the result of a different balance between the elastic recoil of the airway walls (which tends to reduce airway caliber) and the elastic recoil of the lung parenchyma (which tends to increase airway caliber) during inspiration and expiration. In healthy or mildly asthmatic adults, DI causes an increase of airway caliber after inhalation of methacholine (4, 7) but a decrease after inhalation of regular doses of β-adrenoceptor agonist (1, 35). These effects are compatible with the notion that airway hysteresis is greater when airway smooth muscle is contracted than when it is relaxed (31). More recently, Fredberg et al. (9) have shown that the changes in airway smooth muscle hysteresivity after activation depend on the amplitude of tidal stretch. They hypothesized that induced bronchoconstriction in healthy individuals may correspond to tidal stretch amplitudes sufficient to result in an increase of airway hysteresis, thus explaining the bronchodilator effect of DI.

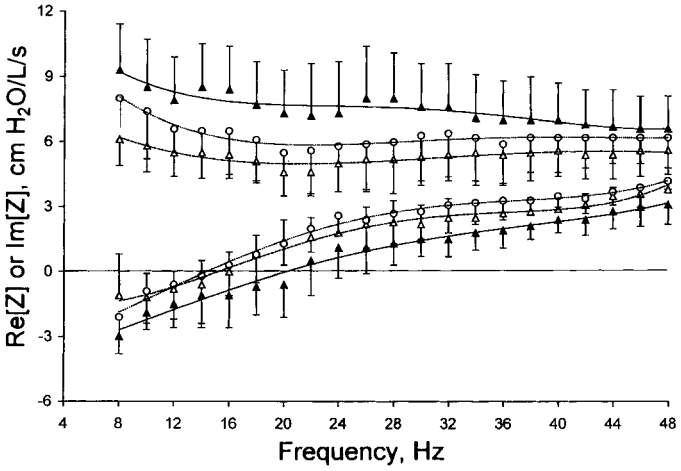

Fig. 4. Re[Z] (*top curves*) and Im[Z] (*bottom curves*) at control (▲, solid lines), after salbutamol (△, dashed lines), and after DI (○, dotted lines). Data points are means ± SD, $n = 9$; lines were obtained by 4th-order polynomial fitting of mean values. Note that DI partially reversed the effect of salbutamol on Re[Z] but not on resonance frequency.

Failure of DI to reverse induced bronchoconstriction may occur because airway and parenchymal hystereses change at the same time and by the same amount, thus maintaining their ratio unchanged (4, 7), or because the distending pressure of lung parenchyma is insufficient to stretch contracted airway smooth muscle or not properly transmitted to airway walls (17). Furthermore, the distending force of lung parenchyma on airways may be ineffective if an active tension develops in the airway smooth muscle in response to DI. In infants, DI delivered by a raised-volume technique did not cause bronchodilatation during methacholine-induced bronchoconstriction (12). Although the distribution of pressures across the respiratory system during passive and active inflations may differ, the lack of effect of DI on airway caliber in infants may be interpreted as suggesting an effect of methacholine on lung parenchyma or an insufficient force of interdependence. Indeed, the elastic recoil pressure of the lung is low at birth (6) and the number of alveoli (and thus alveolar attachments to bronchial walls) is small (2). In asthmatic adults, atropine failed to restore the ability of DI to reverse induced bronchoconstriction (8), suggesting that vagal reflexes are not the cause of impaired bronchodilator effect of DI. Whether reflex or myogenic (19, 26) mechanisms increasing airway smooth muscle tone in response to DI occur in infancy is, however, unknown.

The results of the present study show that, at an age when the number of alveoli is probably close to that of adulthood (2), DI is able to induce changes in airway

caliber that are similar in direction to those observed in healthy and mildly asthmatics adults, i.e., bronchocodilatation during drug-induced bronchoconstriction and bronchoconstriction during drug-induced bronchodilatation. At control, DI caused slight bronchoconstriction in the whole group, when average baseline resistance was low, but slight bronchodilatation in the subgroup of children tested when their baseline resistance was increased. These different effects of DI at control can be accounted for by spontaneous variations in airway smooth muscle tone and are consistent with the results observed during induced bronchoconstriction and induced bronchodilatation. Altogether, the results of the present study are compatible with the relative hysteresis theory (10) and suggest that airway-to-parenchyma interdependence was operative in these children.

There was a gender-related difference, in that the bronchodilator effect of DI was much greater in girls than in boys. This difference, which is consistent with previous findings in older children (15), may be related to different growth patterns of airways and lung parenchyma between girls and boys (23), and it might contribute to a greater severity of asthma in the latter.

There are a number of other mechanisms that could be invoked to explain the effects of DI on airway caliber. The size of the laryngeal aperture may change after DI, thus affecting total respiratory resistance. Laryngeal resistance increases after DI during methacholine-induced bronchoconstriction but decreases in the absence of it (32). Therefore, such changes in laryngeal resistance cannot explain the findings of the present study. An increase in respiratory resistance after DI could be the result of an increase in airway smooth muscle tone, either by a reflex (11) or a myogenic (19, 26) mechanism. However, it can be expected that any effect involving an increase of airway smooth muscle tone is reduced in the presence of salbutamol, which is opposite to the present findings.

An intriguing observation of this study was that $Re[Z]_{12-16}$ and resonance frequency changed consistently after the pharmacological interventions but not always after DI. The increase of $Re[Z]_{12-16}$ induced by methacholine was associated with an increase of resonance frequency, which could be interpreted as the result of a decrease in tissue compliance, an increased inhomogeneity within the lung, or both (29). Indeed, airways may become stiffer and uneven distribution of lung mechanic properties may occur in both induced (21) and spontaneous bronchoconstriction in asthma (13). Conversely, the decrease of resonance frequency associated with the reduction of $Re[Z]_{12-16}$ induced by salbutamol may reflect an increase in tissue compliance, a decreased inhomogeneity within the lung, or both. After DI, the resonance frequency decreased with the decrease of $Re[Z]_{12-16}$ when airways were constricted. This may be interpreted as suggesting an increased tissue compliance, as a result of airway smooth muscle stretching (9), or a decreased inhomogeneity, as a result of a greater dilator effect of DI on the more constricted airways. During salbutamol-induced bronchodilatation, DI increased $Re[Z]_{12-16}$ without affecting resonance frequency. It is conceivable that DI may decrease the caliber of airways dilated by salbutamol without causing an increase in airway smooth muscle tone, thus leaving tissue compliance unaltered. On the other hand, the constrictor effect of DI may be evenly distributed within the lung after salbutamol, thus leaving lung homogeneity unaltered.

In conclusion, the results of the present study show that, in 4- to 7-yr-old children suffering from recurrent wheezing, DI can substantially affect airway caliber both at control condition and after pharmacological interventions (either induced bronchoconstriction or induced bronchodilatation). A practical inference from these findings is that the effects of volume history should be taken into account, particularly when a seemingly simple method such as a forced-oscillation technique is employed.

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