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Attenuation of induced bronchoconstriction in healthy subjects: effects of breathing depth

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Salerno, Francesco G., Riccardo Pellegrino, Gianluca Trocchio, Antonio Spanevello, Vito Brusasco, and Emanuele Crimi. Attenuation of induced bronchoconstriction in healthy subjects: effects of breathing depth. *J Appl Physiol* 98: 817–821, 2005. First published October 8, 2004; doi:10.1152/japplphysiol.00763.2004.— The effects of breathing depth in attenuating induced bronchoconstriction were studied in 12 healthy subjects. On four separate, randomized occasions, the depth of a series of five breaths taken soon (∼1 min) after methacholine (MCh) inhalation was varied from spontaneous tidal volume to lung volumes terminating at ∼80, ∼90, and 100% of total lung capacity (TLC). Partial forced expiratory flow at 40% of control forced vital capacity ($\dot{V}_{part}$) and residual volume (RV) were measured at control and again at 2, 7, and 11 min after MCh. The decrease in $\dot{V}_{part}$ and the increase in RV were significantly less when the depth of the five-breath series was progressively increased ($P < 0.001$), with a linear relationship. The attenuating effects of deep breaths of any amplitude were significantly greater on RV than $\dot{V}_{part}$ ($P < 0.01$) and lasted as long as 11 min, despite a slight decrease with time when the end-inspiratory lung volume was 100% of TLC. In conclusion, in healthy subjects exposed to MCh, a series of breaths of different depth up to TLC caused a progressive and sustained attenuation of bronchoconstriction. The effects of the depth of the five-breath series were more evident on the RV than on $\dot{V}_{part}$, likely due to the different mechanisms that regulate airway closure and expiratory flow limitation.

deep breath; airflow obstruction; partial forced expiratory flow; residual volume

SEVERAL STUDIES HAVE DOCUMENTED the ability of reversing induced airway narrowing by deep breaths in healthy humans (4, 5, 7, 14, 18, 23–25, 31, 35, 38). This effect has been inferred from the increase in forced expiratory flows or the decrease in airway resistance or residual volume (RV) when a full lung inflation was taken after exposing the airways to a constrictor agent. This phenomenon is basically absent or modest in asthma and chronic obstructive pulmonary disease and may contribute to explain the degree of airway hyperresponsiveness (2, 4, 5, 14, 23–25). The understanding of the mechanisms regulating airway tone in healthy conditions becomes central to the knowledge of the pathophysiology of obstructive lung diseases. All studies have so far focused on the effects of deep breaths taken to total lung capacity (TLC), but little is known about the effects of submaximal inspirations. The only few studies that addressed this issue were conducted in animals after exposing the airways or isolated airway smooth muscle to a constrictor agent and documented a progressive decrease in airway resistance or decrease in airway smooth muscle force with gradual increments of the depth of lung inflations (11, 32).

The present study was conducted in healthy humans to examine the effects on airway function of a series of five breaths of variable depth taken soon after inducing airway narrowing by methacholine (MCh). It was intended to address three main questions. First, is induced airway narrowing progressively attenuated by increasing the amplitude of the breaths, or is there a threshold for the effects of lung inflation to become apparent? Second, for expiratory flows and RV determined by different mechanisms and at different sites of the bronchial tree (13, 27), are they differently affected by the depth of breaths? Finally, are the bronchodilator effects resulting from submaximal and maximal deep breaths lasting for similar or different times?

METHODS

Subjects. Twelve healthy, nonsmoker volunteers were studied (Table 1) after giving written, informed consent, as approved by the local Ethics Committee.

Lung function measurements. Spirometry and lung volumes were measured by a Vmax 22 system with an Autobox V6200 (SensorMedics, Yorba Linda, CA). Mouth flow was measured by a mass flow sensor, and volume was obtained by numerical integration of the flow signal. Thoracic gas volume at functional residual capacity (FRC) was measured with the subjects sitting in the plethysmograph and panting against a closed shutter at a frequency slightly <1 Hz. After opening the shutter, subjects were asked to inspire to TLC and then expire forcefully to RV. This allowed measurement of forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC), according to the recommendations of the American Thoracic Society (1). TLC was calculated by adding the linked inspiratory capacity to FRC, and RV was obtained by subtracting FVC from TLC.

On a prestudy day, each subject underwent a standard inhalation challenge to determine the dose of MCh causing an FEV_1 decrease ≥10% from control, which was to be used as a single dose on study days. Solutions of MCh were prepared on the same day by adding distilled water to powdered MCh chloride (Laboratorio Farmaceutico Lofarma, Milan, Italy). Aerosol was delivered during quiet breathing by an SM-1 Rosenthal Breath-Activated Dosimeter (SensorMedics). After 10 inhalations of saline, MCh was administered with twofold increments of dose until an FEV_1 decrease ≥10% of control was achieved. Dose increments were obtained by using MCh concentrations of 1 and 10 mg/ml with appropriate numbers of inhalations.

Table 1. *Subjects' characteristics*

Age, yr	33 (10)
Sex (male/female)	9/3
Height, cm	171 (10)
BMI, kg/cm^2	23 (5)
FEV$_1$, %predicted	102 (4)
FVC, %predicted	99 (6)
TLC, %predicted	98 (3)
FRC, %predicted	102 (9)
RV, %predicted	108 (13)

Values are means (SD). BMI, body mass index; FEV$_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume. Predicted values are from Quanjer et al. (28).

Study protocol. The study was conducted on 4 separate study days in a random order. On each study day, control measurements of lung function were obtained in triplicate. Then the predetermined single dose of MCh was inhaled. Forty-five seconds after the end of inhalation, the subjects had their FRC measured and then were asked to take a series of five breaths, the depth of which varied among study days (see below). Single-lung function measurements were obtained at 2, 7, and 11 min after the end of MCh inhalation.

To avoid any effect of volume history connected with lung function measurements, forced expiratory flow was measured in the body plethysmograph during a forced maneuver initiated from end-tidal inspiration and terminated at RV after at least 6 s. Estimation of FRC immediately before the maneuver allowed partial flow to be measured at 40% of control FVC ($\dot{V}_{part}$) and RV to be assessed as absolute volume during each step of the study.

The depth of the five breaths taken after MCh inhalation was varied among study days by terminating inspirations at the following lung volumes: *1*) end-tidal spontaneous inspiration, *2*) ~80% of TLC, *3*) ~90% of TLC, and *4*) 100% of TLC. Each of these volume points was determined by adding a tidal volume of suitable depth to the FRC measured 45 s after the end of MCh inhalation. A computer screen was used as a feedback to help subjects to attain the target end-inspiratory lung volumes (EILV). Each five-breath series was completed in ~15 s.

Data analysis and statistics. The effect of the depth of breathing on MCh-induced changes in $\dot{V}_{part}$ and RV was tested for significance in each individual by linearly regressing their values against the EILV (as percentage of TLC) achieved with the five breaths taken after MCh on the different study days. To compare the relative effects of deep breaths on $\dot{V}_{part}$ and RV, an attenuation index was calculated at each time point as the ratio of change with to without deep breaths after MCh. Differences between study days at individual time points were tested by repeated-measures ANOVA and Duncan's post hoc test (Computerized Statistical Software, CSS, StatSoft 1991). $P < 0.05$ was considered statistically significant. Values are reported as means (SD).

RESULTS

At control, there were no significant differences in $\dot{V}_{part}$ and RV between study days (Table 2) or within subjects.

On the study day when no deep breaths were taken after MCh, $\dot{V}_{part}$ decreased at 2 min by 94% (SD 5) ($P < 0.001$), and RV increased by 64% (SD 32) ($P < 0.001$). The changes remained fairly stable up to 11 min (Table 2 and Fig. 1).

On any study day when MCh inhalation was followed by a five-breath series of increasing depth, all $\dot{V}_{part}$ values were greater ($P = 0.0001$) and RV values smaller ($P < 0.0001$) than when no deep breaths were taken (Table 2). With the five-breath series at 80 and 90% of TLC, both RV and $\dot{V}_{part}$

remained stable up to 11 min. By contrast, with the five-breath series at 100% of TLC, the RV increased and $\dot{V}_{part}$ decreased slightly at 7 min ($P = 0.006$ and $P = 0.014$, respectively) and 11 min ($P = 0.022$ and $P = 0.043$, respectively) compared with 2 min, thus suggesting renarrowing over time (Fig. 1 and Table 2).

The mean regression slopes of the percent changes in $\dot{V}_{part}$ and RV against the end-inspiratory volumes of the five-breath series were significantly different from zero for all time points (Table 3), indicating progressive bronchodilator effects with increasing breathing depth.

The attenuation index for RV was significantly greater than for $\dot{V}_{part}$ ($P = 0.005$) at any time point and for any depth of the five-breath series (Fig. 2), thus suggesting that deep breaths were more effective in reversing the increase of RV than the decrease of $\dot{V}_{part}$.

DISCUSSION

The main findings of the present study are that *1*) healthy subjects were able to attenuate induced bronchoconstriction (decrease in $\dot{V}_{part}$) and air trapping (increase in RV) in a manner that was directly and linearly related to the depth of a five-breath series taken soon after MCh, *2*) for any depth of the five-breath series, the attenuating effect on RV was more pronounced than on $\dot{V}_{part}$, and *3*) the effects of deep breaths series on lung function lasted as long as 11 min, except with the five breaths at 100% of TLC when slight but significant airway narrowing reoccurred with time.

Since the first observation made by Nadel and Tierney in 1961 (18), it became apparent that deep breath is a very potent mechanism capable of reversing bronchoconstriction induced in healthy humans by pharmacological or physical stimuli. The numerous papers that followed had the merit to elucidate the phenomenon by exploring the underlying mechanisms (4, 5, 7, 10, 14, 18, 23–25, 31, 35, 38). In this regard, the theory that has gained the best scientific evidence is based on the concept that the negative pleural pressure generated by a full contraction of the inspiratory muscles is transmitted to the external surface of the intrathoracic airways through the interdependence with

Table 2. *Partial expiratory flow at $\dot{V}_{part}$ and RV at control and specific time points after single dose of methacholine*

EILV	67% (6) TLC (Spontaneous Breathing)	80% (2) TLC	91% (2) TLC	100% TLC
Control				
$\dot{V}_{part}$, l/s	3.14 (0.97)	2.94 (0.84)	3.00 (0.98)	2.86 (1.10)
RV, liters	1.85 (0.22)	1.86 (0.27)	1.97 (0.24)	1.92 (0.32)
2 min				
$\dot{V}_{part}$, l/s	0.17 (0.11)	0.38 (0.23)	0.49 (0.35)	0.63 (0.25)
RV, liters	3.02 (0.66)	2.55 (0.61)	2.64 (0.75)	2.34 (0.55)
7 min				
$\dot{V}_{part}$, l/s	0.16 (0.08)	0.34 (0.20)	0.42 (0.34)	0.48 (0.26)
RV, liters	2.96 (0.68)	2.60 (0.52)	2.76 (0.71)	2.51 (0.49)
11 min				
$\dot{V}_{part}$, l/s	0.19 (0.11)	0.35 (0.20)	0.45 (0.26)	0.56 (0.32)
RV, liters	2.92 (0.64)	2.57 (0.57)	2.68 (0.63)	2.45 (0.56)

Values are means (SD). EILV, end-inspiratory lung volume of a series of 5 breaths taken ~1 min after methacholine, as percentage of TLC; $\dot{V}_{part}$, partial expiratory flow at 40% of control FVC. At any time point after methacholine and for any breath depth, $\dot{V}_{part}$ and RV were significantly different from control.
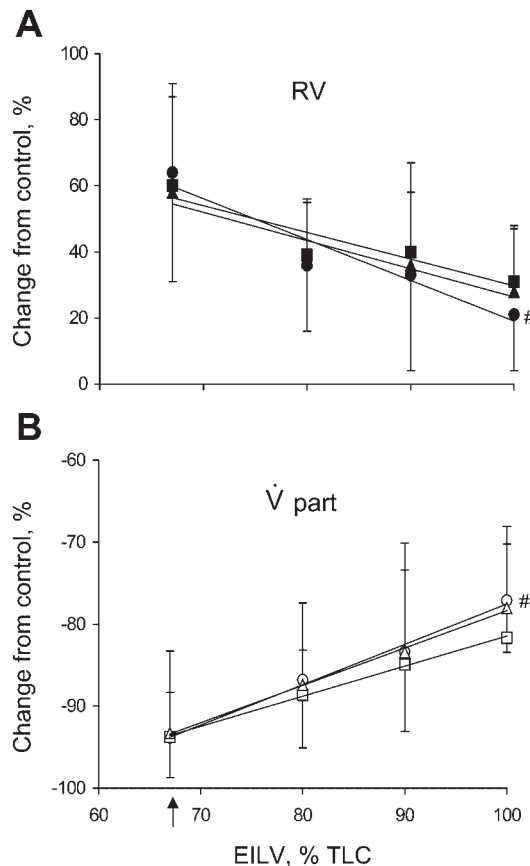
Fig. 1. Increase in residual volume (RV; *A*) and decrease in partial flow ($\dot{V}_{part}$; *B*) as a function of the depth of breathing. EILV, %TLC: end-inspiratory lung volume as percentage of total lung capacity attained during a 5-breath series taken over 15 s soon after methacholine (MCh). Arrow indicates the average EILV during spontaneous tidal breathing. Measurements were obtained at 2 (circles), 7 (squares), and 11 (triangles) min after MCh. Note the different scales for RV (*A*) and $\dot{V}_{part}$ (*B*). Values are means (SD). #$P < 0.05$ for 2 vs. 7 and 11 min.

lung parenchyma (14, 23). From here, the physical stress is applied to airway smooth muscle where it may critically affect the actin-myosin kinetics (8, 9), the organization of the contractile apparatus within the cytoskeletal scaffolding (12, 26), or both. Owing to the exponential relationship between lung volume and transpulmonary pressure (Ptp), the stress applied to the airway walls should increase nonlinearly with the depth of breaths and possibly become more appreciable when end-tidal inspiration approaches TLC.

In keeping with previous animal studies (11, 29, 30, 32, 33), this is the first human study showing that induced bronchoconstriction can be significantly attenuated even by moderate increments of tidal excursions, and this effect is linearly related

Table 3. *Mean linear regression slopes of $\dot{V}_{part}$ and RV against the EILV of 5-breath series taken at 2, 7, and 11 min after inhalation of methacholine*

	2 min	7 min	11 min
$\dot{V}_{part}$	0.48 (0.17)†	0.34 (0.15)*	0.45 (0.25)*
RV	−1.21 (0.68)†	−0.84 (0.68)*	−0.90 (0.52)†

Values are means (SD). *$P < 0.01$ and †$P < 0.001$ vs. 0.

to their amplitude. In isolated airway smooth muscle, the contractile force of airway smooth muscle decreases almost linearly with the amplitude of the applied tidal stretch (8). We are well aware that changes in $\dot{V}_{part}$ and RV are only indirectly related to changes in airway smooth muscle force, and the observed linear regression of RV and $\dot{V}_{part}$ against the EILV of post-MCh breaths cannot definitely prove that airway smooth muscle force is modulated in vivo by the amplitude of tidal stretching rather than Ptp. Yet two additional elements suggest that this could be the case. First, in a subject who accepted to have lung mechanics measured via an esophageal balloon on all study days, dynamic lung elastance measured after MCh was related linearly to the EILV but exponentially to the Ptp achieved with the five-breath series (Fig. 3). Second, neither area and pressure of intact bronchi (17) nor tension and length of isolated airway smooth muscle (37) are linearly related to each other. Thus, if the effect of the high Ptp at maximum lung volume on airway caliber is presumably offset by a relevant increase in airway wall stiffness, then the primary factor modulating airway smooth muscle tone in vivo appears to be the volume rather than pressure change to which the airways are exposed.

Another novelty of the present study is that the effects of any five-breath series of depth greater than spontaneous tidal breathing were more pronounced on RV than $\dot{V}_{part}$. It can be speculated that this may be due to the different mechanisms regulating maximal flow and volume. For flow, the maximum value is determined at the level of the central airways where choke points are located and tend to remain through most of the
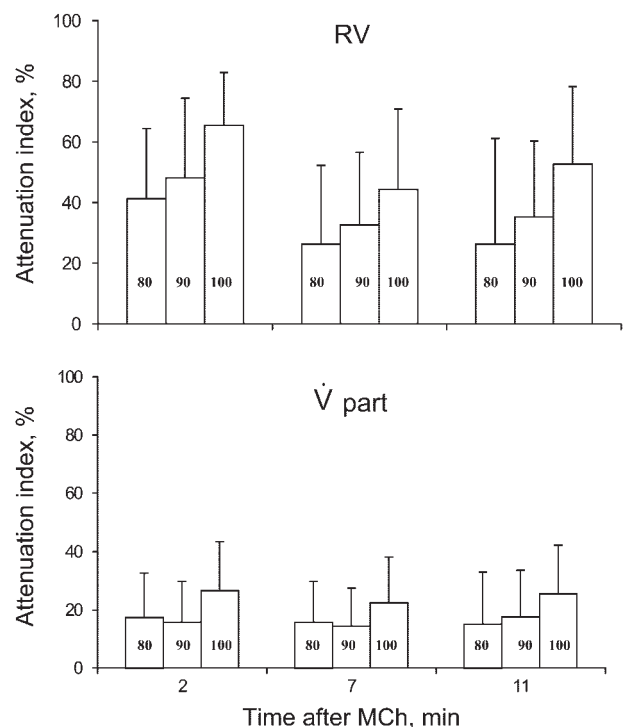

Fig. 2. Attenuating effects of deep breaths on changes in RV (*top*) and $\dot{V}_{part}$ (*bottom*). The attenuation index was calculated by dividing percent changes with deep breaths by percent changes with spontaneous tidal breaths. Nos. within bars indicate the EILV as %TLC attained during the 5-breath series. Values are means (SD). The attenuating effect on RV was significant greater than that on $\dot{V}_{part}$ at all EILV and any time point ($P < 0.01$).
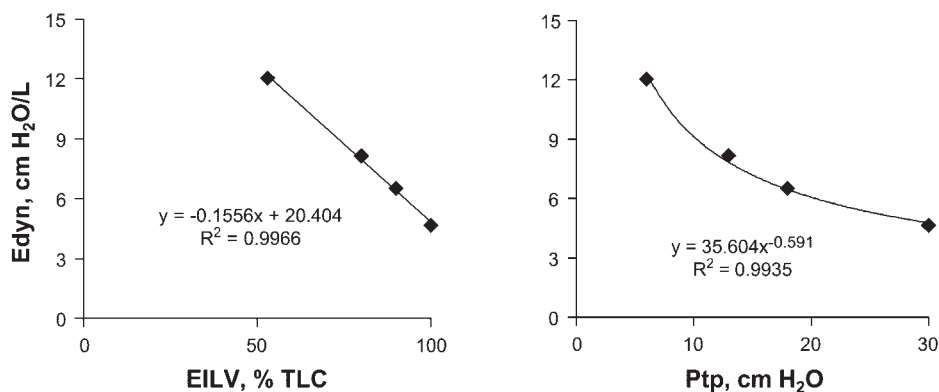
Fig. 3. Dynamic elastance (Edyn) measured in 1 subject at 2 min after MCh plotted against the EILV and the corresponding transpulmonary pressures (Ptp) of each 5-breath series. Edyn was measured during tidal breathing with standard esophageal balloon technique (22, 25). Flow was measured by a Hans-Rudolph pneumotachograph with a Validyne differential pressure transducers (± 5 cmH$_2$O, 0–400 l/min). Ptp was the difference between esophageal and mouth pressures measured by two DP-15 Validyne differential pressure transducers (± 150 cmH$_2$O). Data were processed by dedicated software (DR9, Raytech Instruments, Vancouver, BC) and ANADAT 5.1 software (RTH InfoDat, Montreal, Quebec). Edyn was the Ptp difference at zero flow between end inspiration and end expiration divided by tidal volume.

forced expiratory maneuver (13, 21, 36). In contrast, the increase in RV during airway obstruction reflects extreme narrowing or closure of small airways (16, 27), even though there are data suggesting that large intraparenchymal airways may occasionally close in asthma during bronchoconstriction (15, 22). Based on the present findings, the following scenario can be envisaged. After exposure to MCh and with no breaths deeper than spontaneous tidal breathing, a number of peripheral airways closed or became extremely flow limited so that RV increased. This likely occurred because the high compliance of small airways (17, 19) impeded them to sufficiently oppose the force developed by the airway smooth muscle. For the same reason, with the increasing of the depth of the five-breath series, more and more small airways were eventually recruited, and their smooth muscle stretched proportionally. Thus the number of small airways remaining closed or closing, again when spontaneous tidal breathing was resumed, was inversely related to the EILV achieved during the series of five breaths at increasing depth. In contrast, the less evident effects of the deep breaths on $\dot{V}_{part}$ than RV might be explained by the low compliance of the large intrathoracic airways (19), which makes them more resistant than small airways to distending forces.

The above interpretation that large breaths result in long-lasting airway reopening is also substantiated by the data of lung mechanics measured in the single subject. The increase in dynamic lung elastance with MCh was progressively reduced at any time point when the depth of the five-breath series was increased, which can be interpreted as reflecting an increase in the number of ventilated lung units. With regard to the possible effect of deep breaths on lung elastic recoil, there were no systematic differences in Ptp between days and time in the single subject in whom lung mechanics was measured, thus reinforcing the idea that deep breaths affected flow and volume by primarily modulating the behavior of the airways rather than the lung elastic properties.

There was a slight increase in RV and a decrease in $\dot{V}_{part}$ at 7 and 11 min compared with 2 min after MCh when the EILV of the five-breath series attained 100% of TLC. This could be explained by the effects of MCh lasting relatively longer than the mechanical effect of deep breaths. However, the interesting question is why and how any series of five large breaths had so long-lasting an effect on $\dot{V}_{part}$ and RV. Most previous studies examined the effects of a single breath rather than a series of breaths of different size on lung function, and only for a relatively limited period of time (2, 20, 25). Indeed, the main findings indicate that a single deep breath taken when the airways are constricted had quite an ephemeral bronchodilator effect, varying from a few seconds to slightly less than a couple of minutes, depending on what parameter is considered (2, 20, 25). In contrast, data in canine isolated airway smooth muscle indicate that it takes almost 8 min for the force to redevelop after an initial stretching (11). In a recent study (6), it was shown that deep breathing on exercise during natural or induced asthma had a bronchodilator effect that was about eight times greater than that of a single deep breath taken at rest. The increase in flow occurred since the first exercise steps and was proportional to the increase in EILV. Altogether, these findings seem to indicate that multiple deep breaths of submaximal amplitude have a great ability to decrease the bronchial tone compared with a single, full, deep breath, even in asthma. We speculate that this may occur as a result of a greater response at the level of the contractile and/or the noncontractile tissues when the airways are repeatedly subjected to continuous mechanical stress.

The above interpretation has been based on the principle that any mechanical force applied to the airways depresses airways smooth muscles activity simply by passively stretching it. However, active mechanisms have been postulated to explain the efficacy and duration of the deep breaths on bronchial tone in healthy humans. Admittedly, nitric oxide could be one of the putative molecules capable of actively reducing airway smooth muscle tone (3, 34), but whether the amount of this compound is differently released in the small compared with the large airways during inflation maneuvers of various depth or whether the airways have different sensitiveness to it is unknown. If this was the case in the present study, then it could be speculated that multiple mechanisms likely concurred to dilate the airways and keep them open after a series of breaths with different sizes. Even within this scenario, however, the extent of the effects of the deep breaths on $\dot{V}_{part}$ and RV would still be basically explained by the different compliance of large and

small airways. Finally, the data of this study do not allow any speculation on whether these findings were affected by the magnitude of the bronchoconstrictor response achieved with the dose of MCh used. Further studies are necessary to elucidate this issue.

In conclusion, the present study reveals previously unknown features of one of the most potent mechanisms regulating airway caliber in healthy subjects. Breaths of variable amplitude are quite effective in modulating induced airway narrowing in humans in an amplitude-dependent fashion. The fact that this is more evident on RV than $\dot{V}_{part}$ suggests that peripheral airways are likely more subjected to airway closure than central airways, yet they can also benefit more from stretching. Finally, the effects of deep breaths of any amplitude are long lasting, possibly suggesting the contribution of active mechanisms in modulating bronchial tone.

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