Relating maximum airway dilation and subsequent reconstriction to reactivity in human lungs

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Submitted 3 November 2003; accepted in final form 18 January 2004

Black, Lauren D., Angela C. Henderson, Haytham Atileh, Elliot Israel, Edward P. Ingenito, and Kenneth R. Lutchen. Relating maximum airway dilation and subsequent reconstriction to reactivity in human lungs. J Appl Physiol 96: 1808–1814, 2004—Measurements of airway resistance (Raw) during deep inspiration (DI) suggest that asthmatic subjects possess more reactive airways. There is evidence that one can enhance airway reactivity in healthy lungs by prohibiting DI for an extended period. First, we determined whether the maximum dilation capacity of asthmatic subjects depended on the rate of the DI. Second, we investigated whether the enhanced reactivity in healthy humans might derive from additional mechanisms not present in asthmatic subjects. For the first goal, we tracked Raw in seven healthy and seven asthmatic subjects during a noncoached DI, a DI with a 5- to 10-s breath hold at total lung capacity, and a rapid DI. We found that the minimum resistance achieved at total lung capacity was independent of the manner in which the DI was performed. For the second goal, we tracked the rate of return of Raw after a DI as well as dynamic lung elastance before and after the DI, at baseline and after bronchial challenge. A drop in lung elastance post-DI would indicate reopening of lung regions and/or reduced heterogeneities. The data show that consricted healthy but not asthmatic subjects produce longer lasting residual dilation. Hence, a portion of the enhanced reactivity in a healthy subject’s response to DI is likely due to airway closure and/or atelectasis that can be ablated with a DI. We conclude that preventing DIs does not ensure that healthy subjects will transition entirely to an asthmatic-like hyperreactive lung state.

A recent study in dogs suggests that the degree of smooth muscle stretching is a function of the rate at which the DI is performed and that constricted airways tended to dilate to their maximum airway area slower than relaxed airways (1). Thus one goal of the present study was to quantify the sensitivity of maximum dilation capacity and rate of reconstriction to the rate and manner in which the DI maneuver is performed. Our first hypothesis was that, after bronchoconstriction, asthmatic subjects have a defect in their capacity to maximally dilate and their airways reconstrict faster, regardless of how the DI is performed. In seven healthy and seven asthmatic subjects at baseline and after a bronchial challenge, we tracked Raw during a DI performed without coaching, a DI with a rapid inspiration, and a DI with a 5-s hold at total lung capacity (TLC).

If asthma is indeed a condition in which the ASM resides in a stiffer and more contractile state, what are the proximal conditions leading to such a state? Several studies on isolated smooth muscle suggest that an environment that prohibits periodic lengthening of the muscle could catalyze a transition of the muscles contractile apparatus to one that is more reactive (4–6, 8). In fact, airway reactivity in healthy lungs can be enhanced by prohibiting the subject from taking a DI for an extended period of time (11, 16). However, in the intact healthy lung, mechanisms such as airway closure and atelectasis could come into play when a DI is prohibited. If so, the same amount of agonist would now be challenging a smaller lung. Moreover, in an airway tree with preexisting closure or atelectasis, the impact of additional random severe constrictions being confined to the remaining lung might amplify the response. The net effect creates the appearance of enhanced reactivity. Conversely, traditional airway stimulants such as inflammation and airway wall remodeling are neither present nor enhanced by prohibiting a DI in healthy subjects. With the techniques of Jensen et al. (9), we can examine with greater resolution the dynamics of airway dilation and reconstriction in healthy lungs prohibited from taking a DI. Hence, our secondary hypothesis was that, after bronchial provocation, a DI can reopen closed airways for an extended period in healthy subjects but not in asthmatic subjects. To test this secondary hypothesis, we repeatedly challenged four healthy subjects over a 45-min period during which they were prohibited from taking any DIs, so as to create a level of airway constriction that was at or near that of spontaneous asthma. We then tracked airway caliber before, during, and after a standard DI maneuver.

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METHODS

Subjects. Measurements were made on seven asthmatic (6 women and 1 man) and seven healthy (3 women and 4 men) subjects, all nonsmokers, before and after a methacholine challenge. Subjects ranged from 19 to 45 yr of age (mean of 26 ± 8 yr for the healthy subject group and mean of 25 ± 7 yr for the asthmatic subject group). Table 1 gives the demographics for the subjects. All asthmatic subjects had been previously diagnosed by a physician according to American Thoracic Society (ATS) guidelines and were currently taking inhaled bronchodilators. Of the seven asthmatic subjects, four used only short-acting β2-agonist (albuterol) and three were on a combination of albuterol and inhaled corticosteroids. Airway hyperreactivity was assessed before the day of the study by interpolating the methacholine dose-response curves to the concentration that causes a 20% decrease from FEV1; M, male; F, female.

Table 1. Subject demographics

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<th>Weight, kg</th>
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<th>PC20</th>
<th>MCh, mg/ml</th>
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FEV1, forced expiratory volume in 1 s; %Pred, percent predicted; PC20, concentration that causes a 20% decrease from FEV1; M, male; F, female.

Experimental measurements. The experimental setup has been previously described in detail elsewhere (9). Briefly, we use a computer-controlled pump to deliver 8-Hz oscillations with an amplitude of 0.9 l/s, which are superimposed on top of the subjects’ normal breathing. Vao is measured by a pneumotachograph (Fleisch no. 2) connected to a differential pressure transducer (Celesco, model LCVR, ±2 cmH2O). Ptp is recorded with a differential pressure transducer (Celesco, model LCVR, ±50 cmH2O) with one tap measuring esophageal pressure via a 10-cm latex balloon catheter inserted into the esophagus transnasally and the other tap measuring airway opening pressure.

During 8-Hz DI maneuvers, there is a three-way valve that is opened that allows the subject to breathe to atmosphere through a high-inertance tube. The high-inertance tubing (~212 ml dead space) behaves as a low-pass filter allowing the patient to breathe at atmosphere. A 5-s hold is made at the airway opening pressure. There is a small O2 bias flow (2 l/min) that ensures that the patient is receiving fresh gas even though the dead space is increased by the tubing. The 8-Hz signal is generated by a computer board (Data Translation, DT-2811 analog-to-digital/digital-to-analog board) at a sampling rate of 100 Hz. The pressure and flow signals are stored digitally on the computer by the same board at a sampling rate of 100 Hz.

Protocol. All subjects first underwent baseline spirometry. A balloon catheter was then placed in the esophagus transnasally. The initial positioning of the balloon was verified with an occlusion test. Each subject was trained on the system for 5–10 min. Once they were comfortable with the system, each subject was asked to perform three different 8-Hz DI maneuvers to probe whether the rate of the DI has any effect on dilation capacity (Fig. 1). During the first maneuver, the standard DI, the subject takes a deep breath in to TLC, then exhales out, and breathes spontaneously. The subject is not coached during this maneuver. Next, the subject is coached to take a deep breath in to
TLC and hold it for 5 s and then exhale and continue spontaneously breathing. In the final maneuver, the subject is coached to inspire rapidly to TLC, exhale passively as before, and once again breathe spontaneously. For each maneuver, the subject is asked to make a tight seal around the mouthpiece with the mouth and firmly support the cheeks. Once set, the subject is asked to breathe tidally for 5–10 breaths, perform the DI maneuver, and then continue breathing tidally for another 5–10 breaths. These three DI maneuvers were done at baseline and after a methacholine challenge.

The methacholine was administered by using a Rosenthal New Standard Dosimeter (Pulmonary Data Services) according to the five-breath dosimeter protocol set forth by ATS. The methacholine dose sequence was 0.078, 0.156, 0.3125, 0.625, 1.25, 2.5, 5, 10, and 25 mg/ml. Healthy subjects were given a modified methacholine challenge. The modified challenge is a methacholine challenge in which DI are prohibited. This type of challenge has been shown to amplify airway hyperreactivity of healthy subjects to a given dose of methacholine (16). In asthmatic subjects, however, the prohibition of DIs has only a minor impact on airway hyperreactivity (2). Thus asthmatic subjects performed a standard methacholine challenge in which basic spirometry was carried out after each dose, and the challenge continued until they reached their PC20 dose.

Extended prohibition of DIs in healthy subjects. Here we examined the effect of 45 min of DI prohibition on airway caliber modulation and the airway’s reconstitution dynamics in healthy subjects. Four healthy subjects were challenged a second time to their PC20 dose and asked not to take any deep breaths for 45 min. The subjects were rechallenged every 15 min to prevent the methacholine’s effect from diminishing. A standard DI maneuver was then performed.

Data analysis. All recorded pressure and flow signals were separately low- and high-pass filtered at a cutoff frequency of 4 Hz (4 Pole Butterworth digital filter) to isolate the 8-Hz from the low-frequency tidal volume changes. The 8-Hz pressure and flow data were sent through the recursive least squares algorithm, and the resulting Raw vs. time data was compensated for both the filter and the algorithm’s phase response (9). Lung volume changes were calculated by integration of the low-frequency flow data. The overall result was Raw vs. lung volume for all subjects, before and after methacholine challenge.

For each tracking data set, two key features were analyzed for all maneuvers: Rmin (the minimum resistance achieved during a DI to TLC) and the Raw at the end-inspiration of each tidal breath post-DI normalized by the mean of the pre-DI Raw. These measurements were used to establish the effects of rate of the DI on maximum airway normalized by the mean of the pre-DI Raw. These measurements were presented as means ± SD. To determine the significance of our findings, t-tests were performed (paired when applicable). Statistical significance was defined as P<0.05.

RESULTS

Dynamic raw data from example subjects. Figure 1 shows an example of the Raw and volume vs. time from a healthy subject for all three maneuvers. Generally, during tidal breathing, the minimum of the Raw occurs at end inspiration and the maximum at end exhalation. However, this is not always the case because glottal artifacts can impose distortions in the measure of Raw, particularly at end expiration. The Raw decreases during a DI until the subject reaches TLC, at which point the resistance is a minimum. This is the case during the standard DI. During the DI hold, every subject reached a minimum Raw on achieving TLC, and this Rmin remained constant throughout the hold. During the rapid-inspiration DI maneuver, there is a slight increase in the Raw at the beginning of the DI, but as the subject approaches TLC the Raw once again decreases to Rmin. This transient increase in the Raw at the beginning of the rapid inspiration DI maneuver is likely caused by a combination of the effects of high peak flows and glottal interference of upper airway patency. We observed some form of this transient in all subjects.

The impact of length of time for DI prohibition and degree of constriction before the DI on the time course of Raw is captured for the example subject of Fig. 2. Let us first look at a typical modified challenge in which a DI is prohibited for ~10 min. Here the healthy subject showed a pre-DI increase in Raw from 2.0 to 3.0 cmH2O·L−1·s, which is still substantially below the pre-DI Raw of 4.5 cmH2O·L−1·s seen at baseline in a typical symptomatic asthmatic subject. On taking a DI, the challenged healthy person reaches an Rmin similar to that achievable at baseline, which is much lower than that achievable by the asthmatic person. Also, during tidal breathing after the DI, the Raw remains low and near that which occurred at baseline before any challenge, i.e., there is a long lasting bronchodilatory effect of the DI. Next, we examine an extended form of the modified challenge in which the same healthy subject refrains from taking a DI for 45 min during repeated administrations of methacholine at the PC20 dose (which was the maximum allowed dose in this case). Indeed, there is an overall increase in bronchoconstriction as evidenced by pre-DI Raw levels increasing to ~4.0 cmH2O·L−1·s, which is nearer to the baseline level of the symptomatic asthmatic subject. Nevertheless, the healthy subject is capable of near-maximum airway dilation, achieving an Rmin close to 1 cmH2O·L−1·s. Perhaps more striking is that the Raw during tidal breathing post-DI remains near the original baseline levels again, i.e., regardless of the pre-DI constriction level, a DI seems to reset the airway calibers to preconstriction levels at least for the five to six breaths over which we have data.

Comparison of pooled data. Figure 3 shows the summary Rmin data comparing the different DI maneuvers for both subject groups before and after challenge. During a standard
uncoached DI, the $R_{\text{min}}$ is similar before and after challenge in healthy subjects. Their $R_{\text{min}}$ at baseline is $0.80 \pm 0.38$ cmH$_2$O·l$^{-1}$·s$^{-1}$, and it is $0.97 \pm 0.28$ cmH$_2$O·l$^{-1}$·s$^{-1}$ after challenge. In asthmatic subjects, $R_{\text{min}}$ is elevated ($P < 0.05$) above asthmatic baseline $R_{\text{min}}$ for all maneuvers, and the 45-min data in healthy subjects are significantly lower ($P < 0.04$) than the asthmatic postchallenge standard DI $R_{\text{min}}$.

**DISCUSSION**

The cardinal feature of asthma is airway hyperreactivity. The origins of hyperreactivity are complex, likely beginning at complex molecular events associated with inflammation and eventually leading to a hypersensitive (i.e., responsive to low dose) airway system capable of excessive narrowing. Recent studies identify that such a hyperreactive airway system likely requires a unique ASM state (5, 8), one that is stiffer, capable of exerting more force if activated, and will display a faster shortening velocity. Consequently, asthmatic subjects’ airways should display a unique response to a DI. Specifically, they should show a diminished capacity to dilate their airways with a DI at baseline, which should be even more diminished after bronchoprovocation. Also, any asthmatic airways that do dilate with a DI should shorten faster than airways that are stretched in healthy lungs. Our previous study (9) tracked airway caliber by measuring airway resistance during a DI and provides evidence of both phenomena. However, we did not control for the rate at which the DI was performed. Hence, the first major goal of this study was to determine whether the maximum dilation capacity of asthmatic subjects depended on the rate of the DI. Additionally, there is evidence that one can enhance airway reactivity in healthy lungs by prohibiting a DI for an extended period. Could prohibition of a DI catalyze a transition of healthy ASM to asthmatic airway muscle conditions, or are other mechanisms in play? Our second major goal was to address this question by examining the maximum dilation and reconstriction dynamics in concert, in healthy subjects constricted to levels similar to symptomatic asthmatic subjects via prohibition of a DI for 45 min.

**Maximum dilation vs. rate of DI.** Maximum dilation capacity was quantified by $R_{\text{min}}$, and three maneuvers were used (Fig. 1). The first was the standard DI maneuver, during which the subject was not coached. This maneuver was essentially the control maneuver in this study. The second maneuver was the DI with a 5-s breath hold at TLC. During this maneuver, the subject was coached to inspire to TLC and then hold the volume there for 5 s, trying to keep the glottis open so that we

*Fig. 3. $R_{\text{min}}$ achieved during each type of DI maneuver averaged over 7 healthy (left) and 7 asthmatic (right) subjects, both before and after a methacholine challenge. Also shown are the results for the 4 healthy subjects who completed the 45-min modified challenge. Note that the postchallenge $R_{\text{min}}$ values are significantly lower ($P < 0.04$) than in healthy subjects for all maneuvers. Also asthmatic postchallenge $R_{\text{min}}$ values are significantly larger in asthmatic ($P < 0.05$) above asthmatic baseline $R_{\text{min}}$ for all maneuvers, and the 45-min data in healthy subjects are significantly lower ($P < 0.04$) than the asthmatic postchallenge standard DI $R_{\text{min}}$.*

*Fig. 4. Ratio of the $R_{\text{min}}$ of each breath post-DI to the mean of the pre-DI $R_{\text{min}}$ averaged over 7 healthy and 7 asthmatic subjects. Shown are plots for asthmatic postchallenge, healthy postchallenge, and healthy post-45-min-challenge.*
could probe the airways. This maneuver was performed to see whether holding a high Ptp for an extended period would allow asthmatic airways to stress relax more, i.e., to ensure we did not underestimate maximum dilation ability. Finally, there was the DI with the rapid inspiration. During this maneuver the subjects were coached to inspire rapidly to TLC and then exhale passively. The idea behind this maneuver was to see whether a transiently high pressure could cause airways to “break” out of the latch state hypothesized by Fredberg et al. (6). We found that Rmin is unaffected by the rate of the DI maneuver and that the rate of reconstriction is the same in healthy and asthmatic subjects at baseline, but postchallenge the healthy subjects showed a longer lasting dilation (on the order of 4–5 breaths).

Jensen et al. (9) found that asthmatic subjects have an impaired ability to maximally dilate their airways with a DI compared with healthy subjects and that this inability to maximally dilate is worse after a methacholine challenge. The data presented here (Fig. 3) show this trend for all three DI maneuvers. Also, none of the different maneuvers could mitigate this diminished dilation capacity. That is, no matter how the asthmatic subject performs the DI, there is no significant improvement or detriment in their ability to maximally dilate with a DI.

We next considered the data from the four healthy subjects who performed a modified challenge while refraining from taking any DIs for 45 min. In all cases, the pre-DI Raw was further elevated from the subjects postchallenge data (see Fig. 2). However, even though their pre-DI Raw was closer to the asthmatic pre-DI Raw, the healthy subjects are still able to get their Rmin down to ~1 cmH2O·l−1·s−1, whereas the asthmatic subject cannot. This data is in agreement with the single-subject data discussed by Jensen et al. (9).

Brown and Mitzner (1) found that, in response to a step increase in pressure, airways that were constricted with methacholine reached their maximum area slower than relaxed airways. They found that the difference in the time it took for the airways to reach their plateau at maximum area was on the order of 6 s. They also reported that the maximum airflow area for the constricted airways was only 78% of the maximum airflow area of the relaxed airways. The results of our study show that a DI with a 5-s hold does not result in further dilation of asthmatic airways from that which occurs in the first few seconds. Hence, stimulated asthmatic airways cannot be fully stretched. Brown and Mitzner also showed that on release of the pressure the constricted airways were slower in returning to their initial area. Our data show that, in terms of tracking the mechanics, the constricted asthmatic subjects’ airways actually return a little bit faster than constricted healthy airways. The differences between our results and those of Brown and Mitzner may lie in the species (human vs. dog), resolution (imaging of a few larger airways vs. near continuous time measures of airflow caliber averaged over the entire airway tree), or experimental methods. With the latter, the Brown and Mitzner study applied effectively an instantaneous change in pressure acting to distend the airways, whereas we relied on the subject to create a distending force voluntarily. More to the point, the differences are not as important as the final result, which is that asthmatic subjects, particularly symptomatic ones, cannot distend the airway lumens as much as healthy subjects.

One possible explanation for the differences in DI response between healthy and asthmatic subjects was put forth by Fredberg et al. (6). They hypothesized that stretching ASM perturbed the binding of actin and myosin, causing a greater number of cross-bridge detachments and thus a less stiff ASM. These results would seem to suggest that the post-DI residual bronchodilation seen in healthy subjects might be attributed to this perturbed myosin binding. Fredberg et al. also suggested that asthmatic ASM may not be stretched enough with tidal breathing and the periodic sigh, causing it to shorten and stiffen to a “latch” state, in which the muscle becomes locked and a even a DI does nothing to break the cross-bridge attachments. Along similar lines, Gunst et al. (7) hypothesized that the increase in stiffness of ASM held at a fixed length compared with ASM subjected to length oscillations was due to a remodeling of the actin cytoskeleton within the ASM. Both of these hypotheses may explain in some part why this difference in reconstriction dynamics exists. However, from our data and those of other studies, it appears that neither of these phenomena occurs with a period of decreased stretch that is on the order of 45 min. That is, preventing healthy subjects from TLC level stretches in airway caliber for 45 min does increase their airway reactivity but does not change their ability to maximally dilate their airways or their post-DI residual bronchodilation.

Rate of reconstriction and airway closure-reopening. With respect to the rate of reconstriction post-DI (Fig. 4), healthy and asthmatic subjects at baseline both returned to 90% of the pre-DI Rmin values in about one or two breaths post-DI regardless of the maneuver. However, after a methacholine challenge, the healthy subjects showed evidence of a longer lasting bronchodilatory effect (3 or 4 breaths) post-DI for all maneuvers, whereas the asthmatic subjects again returned to 90% of the pre-DI Rmin values within one or two breaths. A very recent study by Salome et al. (15) using a technique based on that of Jensen et al. (9) showed that asthmatic subjects had not only a diminished capacity to maximally dilate but also had greater renarrowing on the first breath post-DI as well as a faster rate of reconstriction. These results seem to be in good agreement with previous studies (9) as well as the data presented here. More generally, in both healthy and asthmatic subjects before and after a methacholine challenge, we found that the minimum Raw during tidal breathing returns to only ~90–95% of the pre-DI values some five or six breaths after the DI (Fig. 4). This means that some amount of the pre-DI constriction was completely ablated by the DI for at least the next five breaths. It is also interesting to compare these results with those of Pellegrino et al. (13), who reported a recovery in total lung resistance after a DI. They reported that both asthmatic subjects and healthy subjects had a drop in Rl with a DI and that the Rl took a longer and similar time to recover from that seen with Raw. However, the Rl at typical breathing frequency necessarily reflects tissue resistance and airway heterogeneities rather than Raw alone. Thus we feel that Rl is not as directly reflective of airway caliber recovery vs. time after a DI.

Most intriguing to us were the data from the four healthy subjects after the 45-min modified challenge. As predicted by Skloot et al. (16) and several studies thereafter, prohibition of a DI amplified the response to bronchoconstriction and more so for a 45-min prohibition compared with a 10-min one (e.g., Fig. 2). However, in these subjects, a DI ablated most of the constriction and seems to have reset the airway caliber to that
at baseline for several breaths thereafter (Figs. 2 and 4). These data suggest that, in healthy subjects, some of the increase in Raw was due to airway closures and/or atelectasis that could be ablated by the DI, and these airways and regions do not reclose quickly. It is possible that, had we acquired data for much longer (say 20–30 breaths) post-DI, the Raw would have eventually increased back to the pre-DI levels, assuming that the effectiveness of the methacholine as a bronchoconstrictor had not diminished. With asthmatic subjects, on the other hand, either closed airways cannot open or they reclose immediately. There is some evidence of this in both subject groups after a challenge because neither group appears to reconstrict to values >95% of their pre-DI R_{min} values.

To further investigate the feasibility of this interpretation, we calculated the dynamic lung elastance during tidal breathing (EL) before the DI and after the DI by fitting the pressure and flow data from the tidal breathing to the model described by Eq. 1. If a DI did not result in a lasting reopening of airways, the EL would be the same pre- and post-DI. However, if reopened airways stayed opened after the DI, the EL would decrease, reflecting a larger fraction of lung tissue participating in breathing. Indeed, Fig. 5B shows that, in asthmatic subjects, the EL increased after a challenge, but a DI, whether performed at baseline or after a challenge, produced only a small reduction in EL. In striking contrast, the longer a healthy subject is challenged, the greater the increase in EL with a DI that took approximately five or six breaths to recover to pre-DI levels. These differences may be methodological, and also Pellegrino et al. did not evaluate how a prolonged period of prohibiting a DI impacts the response.

Our EL data suggest that amplified reactivity via prohibition of a DI in healthy people seems partly a consequence of heterogeneous airway closures and/or severe narrowings that are occurring during the course of not taking any deep breaths while being challenged. These closures and narrowings prevent effective communication to distal tissue units during tidal breathing, and, although they are easily ablated with a DI in healthy subjects, they are not so in asthmatic subjects and take a while to reclose (i.e., ≈5 breaths). In contrast, airway hyperreactivity in asthma appears to result from a complex interaction between a stiffer, more contractile ASM and what is likely a local physical and mediator environment distinct from healthy subjects. A DI cannot open up closed airways and cannot reduce R_{min} as much. Any airways that were stretched immediately reconstrict to their pre-DI levels. This implies that the amplified reactivity discovered in the original Skloot study (16) may not have been entirely due to a change in the behavior of ASM, per se and that repetitive challenges of an increasingly smaller lung also contributed. Eventually, a DI opens all the closed airways and airway behavior is again much closer to normal.

In conclusion, the diminished ability of asthmatic subjects to maximally dilate their airways persists regardless of how the DI is performed. Also, although both subject groups appear to have the same reconstriction dynamics at baseline, the healthy subjects show some evidence of a longer lasting residual dilation postchallenge. It is apparent from the rate of reconstriction data that there is some amount of constriction that is completely ablated by the DI in both subject groups at baseline and after a challenge (on the order of 5–10% of their pre-DI constriction). This effect was even more apparent in the healthy subjects who performed the 45-min modified challenge, suggesting that a substantial portion of the increase in healthy subjects’ response to prohibition of DIs is likely due to airway closure and/or atelectasis. Finally, our data show that preventing DIs for 45 min did not cause healthy subjects to transition entirely to an asthmatic-like state. The question then is whether the conjecture by Skloot et al. (16) is an overinterpretation in that mechanisms (e.g., atelectasis and closures) other than reduced length cycling of ASM seem to be also serving to enhance reactivity in the healthy subjects. We point out that our study did not include a large enough number of subjects to confirm this point and does not rely on explicit visualizations confirming the role of closures. Nevertheless, our data imply that the difference in the biological state of the asthmatic subjects’ ASM requires not only a prolonged period of decreased stretching but additional unique stimuli or environments, such as endogenous inflammatory mediators or remodeling.

**GRANTS**

This work was supported by National Heart, Lung, and Blood Institute Grant HL-62269 and the National Science Foundation-Bioengineering Division.
REFERENCES


